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PROJECT COMPLETION
REPORT NO. W119 and 373X
JULY 1967 - JUNE 1970

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United States Department
of the Interior

CONTRACT NO.
B-013-OHIO MG 14-01-0001-1537
B-018-OHIO MG 14-31-0001-3114

State of Ohio
Water Resources Center
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November, 1971

This study was supported in part by
the Office of Water Resources Research,
U. S. Department of the Interior under
Projects B-013-OHIO and B-018-OHIO.

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## Introduction

Increased understanding of the complex process of eutrophication will lend to more suitable control by biological or bioengineering techniques. The eutrophication process in Lake Erie has been stimulated in recent years, and can be attributed to the increasing amounts of nutrients that are being passed into the lake. These nutrients accumulate from major rivers and runoff waters from the water sheds that enter into the lake. The major nutrients involved in this process are carbon, phosphorous and nitrogen. In recent years, phosphorous has been considered a major and a limiting contribution nutrient with respect to algal bloom stimulation and the total process of eutrophication. Nitrogen and carbon are also required in the process and are major elements needed to build microbial or algal protein. In the case of certain fresh water lakes, where tremendous quantities of nutrients (carbon, etc.) are being passed into the lake, eutrophication can be stimulated by the conversion of these nutrients into protein through the intervention of heterotrophic bacteria. This acts as a source of primary production and the presence of high quantities of organic carbon with the increasing presence of the microbial population may in turn stimulate higher yields of algal blooms through the process of respiration. Carbon dioxide released into the water and present as dissolved carbon dioxide is essential for the stimulation of algal blooms. Therefore, the role of the microorganism (non-photosynthetic) in the stimulation of the eutrophication process in certain fresh waters is of maximum importance.

The carbon cycle in such lakes as Erie should be studied very closely, particularly on a microbial level. The biogeochemical cycle of carbon as occurring in lakes, rivers and oceans is especially important in areas where an aerobic and an anaerobic zone are known to occur, that is, in deeper waters or in mud, as exists in Lake Erie.

The fate of organic compounds can be different when they are broken down aerobically or anaerobically. Organic materials which fall into the anaerobic zone are fermented primarily by bacteria with the production of organic acids, methane, hydrogen gas and $CO_2$. After a while, the accumulation of organic acids and other products will lead to an inhibition of microbial action. At this point, the decomposition of organic material stops and organic material can accumulate at the bottom in the mud. This is an alternate energy storage mechanism, similar to the case of coal or oil formation. In the aerobic zone of the water, decomposition generally goes to a complete conversion to $CO_2$. In either case, aerobic or anaerobic, $CO_2$ is released into the surrounding water.

The production of methane by the methanogenic bacteria and the subsequent utilization of methane by methane-oxidizing bacteria also contributes to the increased $CO_2$ level. By increasing the amount of organic material in a body of water either through release of material via anaerobic processes from the bottom or the increased release of $CO_2$ through aerobic processes which becomes dissolved in water, there is a stimulation, or a potential stimulation of algae and other green plants in the light portions of the water. In order to convert or fix some of the $CO_2$ that is dissolved in the water, sunlight is required. In quiescent

periods of the summer, warmer water and long periods of sunlight increase the fixation of $CO_2$, and at the same time increase the rate of primary production at the surface levels of aquatic bodies.

Another important aspect of the carbon cycle is the influence of various forms of carbon in the water and the microbial action on the substances. Classically, the precipitation of calcium carbonate has been of immense geological importance since it leads to the formation of limestone. Calcium carbonate is being precipitated in large amounts in warm waters as well as in lakes, and the role of the living organism has been well established. For example, many aquatic animals have calcium carbonate shells.

To understand the role of microorganisms in calcium carbonate precipitation, it has to be recognized that natural waters are frequently super-saturated with calcium ions that are held in solution as calcium bicarbonate or calcium sulfate. If the pH is changed or the bicarbonate ion concentration is changed, microorganisms can cause calcium carbonate to precipitate. The equilibrium between calcium bicarbonate and calcium carbonate is influenced by the $CO_2$ content of the water, and algae can cause the precipitation of calcium carbonate by removing $CO_2$ during photosynthesis. In addition, there is a very strong relationship of carbon to other nutrient elements such as nitrogen, phosphorous, and trace minerals. Under any given set of particular circumstances, these elements or possibly trace minerals could be considered limiting toward the production of protein. Another very important contributing aspect

to the study is the role of the microparticulate and its interactions with those chemicals which are soluble, in addition to interactions with the particulate material in the lake that is biological.

During recent studies, we have established important relationships with respect to microbial activity and microparticles as well as important associations of hydrocarbons and microparticles in Lake Erie. We have learned that pesticides may become associated (adsorbed) with microparticles, and that these pesticides might even be adsorbed to specific types of particles. Associated pesticides exerted a strong influence on microbial activity, whether in pure form or when attached to the microparticles. Therefore, we are concerned about the increasing concentration of such compounds in the water with the realization that microbial activity is being affected, and that this in turn is going to affect the carbon balance in the lake. We have learned that microparticles can be separated by either density or size differences and that different types of microparticles may have different effects on the microbial population.

The presence of endrin has been shown to cause the production of the lipid-like storage material poly-beta-hydroxybutyrate in some of the Pseudomonas microorganisms isolated from Lake Erie. There is an involvement of oxygen and the action of the pesticides, and therefore the extent to which a lake may be either aerobic or anaerobic could be extremely important with respect to these compounds.

During the past two summers, we have learned that there is a great deal of biological nitrogen fixation in the water as well as the sediments in Lake Erie. This biological nitrogen fixation was determined by the acetylene reduction technique. Nitrogen fixing in the water activity occurred throughout the months of August through November and in the sediments throughout the year, suggesting that it may be significant over the extremes of seasonal variation in light, temperature and nutrients. In addition, we have clearly established a nitrogen fixing potential in the sediments of the lake as well. This potential is essential when consideration of the analysis of eutrophication in this body of water is made. We have learned in the past two summers that immense quantities of methane are produced in the areas of the lake where sediments occur. We have also learned that there are methane oxidizing bacteria present in this body of water as well. The activity in the sediments is important with respect to the release of nutrients into the water above. Carbon dioxide, $N_2$, $CH_4$, etc., are released in this process, and are involved in the carbon balance of the lake.

Many heterotrophic bacteria capable of forming flocs have been isolated. Floc formation in such systems is important for typing up nutrients, and minerals and bringing them to the bottom of the lake. We have established that chlorinated hydrocarbon compounds such as aldrin are rapidly adsorbed to microbial flocs and are easily removed to the bottom muds of the lakes. There appear to be higher concentrations of pesticide in the floc or fuzz on the bottom of the lake.

We have also conducted an analysis of the physiological types of microorganisms (heterotrophic) in the lake over the past three years. This population has been examined from a biochemical, physiological standpoint and analyzed using a numerical computer technique. We have learned some interesting facts concenring the alteration and change of microbial population with respect to time and feel that we must learn more to properly evaluate the role of the microorganism in the total eutrophication process.

## Literature Review

From a review of the literatures, it can be concluded that not much data have been collected regarding particulate material (detritus) and microbial systems in the aqueous environment. It is evident that the microscopic particulate matter of natural waters consists of a mixture of living organisms, dead detrital material, and soluble organic and inorganic components, the relative proportions of which probably vary widely. The chemical composition of these fractions appear to be highly variable (Menzel and Ryther, 1964). The composition of non-living matter (detritus) depends on its age and stage of its decomposition or mineralization. In the euphotic zone, phytoplankton pigments are rapidly decomposed following death of the organisms (Yentsch, 1962). Phosphorous also is quickly liberated from dead material, while nitrogen and carbon are both relatively refractory to decomposition (Harvey, 1960). The method which has been used to collect particulate matter is principally that of microfiltration and seems to have precluded the possibility of mechanically separating living and dead fractions. Fluorescent microscopy using acridine orange differentially stains living and dead material

which lends itself to a rapid analysis (Ferguson Wood, 1955). Methods are needed in quantitative ecology for the measurement of biomasses of these types. It is for this reason that simple chemical analysis for any single component does not provide a reliable index of these parameters (Menzel and Ryther, 1964). Anthony and Hayes (1964), studying water and sediments of North American lakes, measured chemical and optical properties of water in relation to bacterial counts. They concluded that no relationship between the organic contents of the sediments and the number of bacteria present could be found. They suggested that bacteria may be independent of this property of their environment. They, also, could be related to bacterial numbers.

Davis (1964) presents evidence for the eutrophication of Lake Erie from phytoplankton records accumulated for 25 years. He shows that the phytoplankton has consistently increased between 1920 and 1963 and that definite qualitative changes have also occurred. These results indicate an increased and rapid eutrophication of the waters of Lake Erie. The water chemistry of this condition was summarized by Beeton (1961). The significance of this is the obvious increase of particulate matter including detritus in the lake water in addition to the phytoplankton. This results from the natural decay of the living matter. Riley, et al. (1964), using samples collected from surface waters in the North Atlantic Ocean and in subtropical areas, filtered the water using millepore filters and counted the number of aggregates per area of filter. Phytoplankton were counted, and the specimens were measured for particulate organic carbon. Their data indicated that most of the particulate organic carbon was non-living. The carbon content

of the phytoplankton was "almost certainly less than 10 percent of the wet weight of cell contents (numerically approximately equivalent to the volume of cells)." They suggested that some of the variance in their results might have been due to fragile forms which were not preserved in recognizable form on the filters. These organisms might constitute the bulk of the population in the poorer waters, that is, the waters where their results were low, but this fraction would be small in a totally large or diatom-rich water. In short, their data did not lead to a precise evaluation of living and non-living organic matter, but they did not doubt that organic aggregates constituted a major fraction. The authors suggest that there is a pool of dissolved organic matter which can be drawn upon to produce particulate matter. Fifteen to twenty liters of water were passed through a millipore filter, and, then, air was bubbled through this water to determine how much additional material could be formed from the filter-passing materials. Their evidence suggested a small, but relatively constant, fraction of adsorbably organic matter and would have far-reaching consequences in the natural environment.

Rodina (1963) has examined the detritus in seven lakes in the vicinity of Lake Lagoda. His investigation was carried out by microscopic examination using visible and ultraviolet light. Total numbers of microbes in the presence of physiological groups were determined. The author showed that the detritus of these lakes was heterogeneous and composed chiefly of dying plankton. He has shown the distribution of bacteria on particles and stated that accumulations of bacteria are components of the detritus. The numbers of microbes in the detritus vary widely and seem

to depend on the predominance of various components in the stage of their decay. Rodina has pointed out, "The significance of detritus in the productivity of water bodies is determined by its role in the formation of an active layer of sediment and by its wide use as food by planktonic and bottom animals. There are few data on the microbiology of detritus." Rodina also pointed out the nutrient significance of detritus, especially for different species of Cladocera. Ultraviolet microscopy revealed that bacteria often surround living algae and that bacteria develop in the mucus excreted by the algae. The author has characterized the components of lake detritus: "(1) dead and dying algae, (2) decomposed remains of algae, (3) decomposed remains of planktonic animals, (4) decayed pieces of epidermis of hydrophytic leaves, (5) enormous quantities of bacteria transforming detritus and cementing its microscopic particles into conglomerants, (6) products of vital activity of bacteria." It is also mentioned that it seems impossible to separate all the bacteria from detrital particles. Much evidence suggests that the variety and total number of heterotrophic bacteria are lower in young and higher in mature detritus. "Even young detritus contains bacteria excreting $H_2S$ in the course of decomposing albuminous substances. The process increases as mineralization progresses. The number of these bacteria arises to tens and hundreds of thousands per gram. The existence of $H_2S$ leads to the development of different sulfur bacteria on particles of detritus which appear to intercept the $H_2S$ as soon as it is excreted. The number of sulfur bacteria in detritus reaches $1.5 \times 10^9$ per gram" (Rodina, 1963).

Riley, et al. (1965) concluded that there are bacteria and filter-feeding animals in deep water which are able to use particulate matter. The authors claim that most of the organic matter exists as aggregate masses rather than detrital fragments. Chave (1965), examining surface sea water in tropical and sub-tropical areas, found many carbonates and minerals of the size 10-50 $\mu$ in diameter suspended in the water. The water contained organic aggregates of similar sizes. Changes in pH and temperature did not affect the composition of the suspended carbonates.

Mullin (1965) fractionated sea water from a variety of locations into 11 size ranges down to a 10-1 $\mu$ fraction using mechanical netting and filtration. His data suggest that the 10-1$\mu$ fraction contributed the **major** portion of the total carbon and, "Was the single richest category." The author discusses the effect of selective pressures of zooplankton feeding effectively on different food sizes. He suggests that, "An increase in the size diversity of available organic particles may permit an increase in the faunal diversity of zooplankton even if the total biomass of particles remains constant." Particle size diversity per se in relation to microbial life is not easy to estimate (Parsons, 1963). There is importance for small particle size with respect to primary production in water, since most of it is carried out by organisms smaller than 10 $\mu$ (McAllister, et al., cited in Mullin, 1963). In further works, McAllister (1969) has investigated the possibilities of using phytoplankton production to estimate zooplankton production.

In order to understand the process of eutrophication, the complex interactions between dissolved nutrients, particulates, and microorganisms must be understood. Relatively little is known about these relationships and their connection with the carbon balance system. While investigating the effects of environmental contaminants on microbial populations, Pfister, Dugan, and Frea (1968), have developed a procedure involving differential and gradient centrifugation to separate various particulate fractions from water samples according to size and density. In samples collected from Lake Erie at a depth of 15 feet, the most common size particle was in the range 0.1 um (Pfister, Dugan, Frea, Randles, Zaebst, McNair, Duchene, and Kennedy, 1970). These fractions were found to exert an influence on growth of several microbes isolated from the Lake.

Fractions were examined for associated chlorinated pesticides using both gas-liquid chromatography and thin layer chromatography (Pfister, Dugan, Frea, 1969). Pesticides were found associated individually with particles of different density. Aldrin and endrin were found associated with less dense fractions consisting of organics, detritus, and microorganisms, while lindane was found on the more dense inorganic portion. Since only particles larger than 0.15 um were involved, the presence of pesticides indicates a true pesticide-particulate association.

Pesticides are known to effect the growth of phytoplankton. The response of 4 species of marine phytoplankton to dieldrin, endrin, and DDT was determined by Menzel, Anderson, and Randtke, (1970). Effects ranged from an inhibition of Cyclotella by all three at concentrations above 1 ppb to Dunaliella which was insensitive to 1000 ppb. Wheeler

(1970), demonstrated the rapid absorption of $C^{14}$-dieldrin by Chlorella. A maximum per-cell level was reached within 6 to 24 hours of exposure. Heterotrophic bacteria isolated from Lake Erie have been grown in the presence of pesticides (Pfister, Dugan, Frea, Randles, Zaebst, McNair, Duchene, and Kennedy, 1970). Of 151 bacteria isolated 55 were stimulated by aldrin, 54 by endrin, and 45 by dieldrin. Forty-six cultures were inhibited by aldrin, 43 by endrin, and 43 by dieldrin. Eighteen cultures were stimulated by all three, 27 inhibited. That pesticides such as aldrin, endrin, and DDT are actually degraded has been documented by Patil, Matsumura, and Boush (1970), with soil microorganisms.

Two floc forming isolates from Lake Erie were shown to concentrate and accumulate aldrin from solution (Leshniowsky, Dugan, Pfister, Frea, and Randles, 1970). Aldrin adsorption took place primarily during the first 20 minutes of contact and then remained nearly constant. In the Bacillus tested, all of the aldrin was recovered from the floc, none from the supernatant. It is possible that in the natural environment, once the pesticides have been sedimented out in the floc, an anaerobic degradation could take place. Hill and McCarthy (1967), found that degradation of chlorinated hydrocarbon pesticides was more rapid under anaerobic than aerobic conditions.

A study by Kokke (1970), has pointed out the significance of pesticides in the adaptation of microorganisms to their environment. The percentage of DDT accumulating bacteria isolated from varying areas was found to increase as the frequency of their contact with pesticides increased. For example only 1 percent of colonies grown from highly chlorinated drinking water accumulated DDT while 80-95 percent of those isolated from nursery soil recently treated with pesticides did. It

was demonstrated that strains which would not initially grow in the presence of 1 ppm DDT could be induced to grow in two stages using smaller amounts.

Although organic compounds such as pesticides exert an influence on the growth of aquatic microflora, the greatest effect is shown by major nutrients - carbon, nitrogen, phosphorus, and necessary trace elements. Seki (1970), has established that in one liter of sea water an average of 9.9 ug of microbial carbon is found on particulate organic matter. In studies of marine sediments using ATP as an indicator of biomass, Ernst (1970), estimated that the organic carbon content of living matter accounts for 0.13 to 1.6 percent of the total organic carbon in the sediments.

The aspect of the carbon cycle in which organic materials are converted to organic acids, methane, and sulfates has been studied by Foree and McCarty, (1970). The first step in the anaerobic decomposition of algae is a conversion of particulate organic matter to soluble form either by the algae themselves or by bacteria. Methane and sulfate were the prominent products of these bacterial fermentations. Methane fermentation produced significant quantities of acetic and proprionic acid while sulfate reduction produced only acetic acid. The toxicity of soluble sulfides to methane bacteria possibly accounted for the fact that no methane fermentation occurred in cultures with initially high sulfate concentrations. It was significant that the rate and extent of anaerobic conversion of algal matter into soluble forms was the same as those observed under aerobic conditions. The soluble acids produced would effect subsequent bacterial activity especially if the pH were

significantly lowered. Since extremely acidic waters are hostile to most life forms, only a few species of microorganisms could be supported. Studies by Dugan, MacMillan, and Pfister (1969), on acid mine water (pH 2.8) found that the predominant bacterial forms were protected by a tough slime and appeared as "streamers". One species of Bacillus isolated produced a heavy coating of this slime when grown under similar pH conditions in the laboratory. Further studies are necessary to determine long range effects of these and other environmental changes on both fluctuation in microbial populations and microbial successions.

Once organic particulate matter is broken down into organic compounds, it becomes available to new groups of organisms for further attack. Bacterial strains isolated from marine algal cultures have been tested for their ability to attack organic compounds (Berland, Bonin and Baestrini, 1970). Amino and organic acids were found to be utilized more than sugars and derivatives. Little study has been done on the role of sediments as a reservoir of carbon forms and methods of their uptake and conversion by microorganisms. Parsons and Strickland (1961), describe a method using $C^{14}$ labeled substances to study heterotrophic uptake of organic solutes and obtain information on enzyme kinetics of substrate assimilation. Wright and Hobbie (1965, A) revised the procedures to give greater experimental flexibility and allow maximum uptake values to be determined. Continuing this research, a possible method of bioassay for glucose using kinetics of substrate uptake rather than growth as a response reaction was developed (1965, B).

An important aspect of the carbon cycle is the role of methane-oxidizing bacteria in carbon conservation. Hutton and ZoBell (1949), isolated methane-oxidizing bacteria from upper layers of marine sediments and found that 40-90 percent of the methane oxidized was converted to $CO_2$. Carbon was also converted to organic matter as bacterial cell substance. Vary and Johnson (1967), demonstrated with carbon balances that under their growth conditions few, if any, products other than cells and $CO_2$ were produced.

Nitrogen availability must be considered in a complete discussion of the eutrophication process. Using acetylene reduction, Howard (1970), Frea, Pfister, and Dugan (1970), determined that the potential for biological nitrogen fixation exists, both in the waters and sediments of Lake Erie. In situ fixation experiments on blue-green algae suggest that this potential is at least partially realized. Nitrogen fixing activity in the water column did not occur in samples collected in June, July, and early August. Activity increased in late August during an algal bloom, disappeared during September, and reappeared in late October. Heterotrophic nitrogen fixation in lake samples has been demonstrated by Breznoik and Harper (1969). Nitrogenase activity and thus nitrogen fixation was found by Stewart, Haystead and Pearson (1969), to occur in heterocysts of the blue-green algae. Studies by Wyatt and Silvey (1969), on axenic cultures of Gleocapsa adapted to nitrogen-free medium demonstrated that nitrogen fixation by blue-green algae is not confined to genera with heterocysts.

The availability of various trace elements is also significant in the distribution of microorganisms. Patrick, Crum, and Coles (1969), demonstrated that the significance of manganese in determining whether diatom or blue-green algal flora would predominate in streams. They found that if the manganese content of the water is adjusted to a few ppb a blue-green and green algal flora of species typically found in organically polluted water is favored. If the content was changed to .28 mg per liter in recycled water, a diatom flora persisted. Further experiments in which nitrogen was added as nitrates or ammonium, and phosphorus as orthophosphate were run varying the nitrogen and phosphorus amounts and ratios. Diatoms continued as the major algal component while the blue-green remained scarce. Increasing nitrogen and phosphorus produced no floral changes in free flowing streams. The final influence of these and other nutrients on the distribution of carbon have not yet been determined.

## Methods and Results

This section is divided into five parts:

(1) The isolation and identification of chlorinated pesticide in association with microparticulates,

(2) The removal of the chlorinated hydrocarbon aldrin from lake water by flocculent bacteria,

(3) Particulate fractions in water and the relationship to aquatic microflora,

(4) The effects of microparticulates and chlorinated hydrocarbons on microorganisms isolated from lake Erie.

(5) Microbial and chemical interactions in Lake Erie: A summary statement.

Part 1.

The isolation and identification of chlorinated pesticide in association with microparticulates.

Abstract

Microparticulates suspended in lake water were collected by continuous centrifugation and either examinated directly or placed on a linear sucrose gradient. Total residue as well as fractions of the centrifuged gradient were extracted with hexane and examined by gas chromatography for the presence of chlorinated hydrocarbon pesticides. Hexane extracts of total residues were also examined by thin-layer chromatography. Lindane and endrin were shown, by gas-liquid chromatography and thin-layer chromatography, to be associated with microparticles of different densities, when gas-liquid chromatography was used, although concentrations were below the detection limits required for confirmation by thin-layer chromatography. Samples taken at different times from different locations in Lake Erie revealed different associations with hexane-soluble electron-capturing compounds.

## Introduction

The ubiquity of pesticides and the significance of their presence in the aquatic habitat has been established. Techniques for identification of pesticides in natural water usually involve extraction from water by adsorption on activated carbon. This particular method has been included in the 1962 USPHS drinking water standards as the method for the determination of organic substances in water (1). In this procedure, up to 5,000 gallons (about 19,000 liters) of water are passed through a column of activated carbon 18 by 3 inches (45.7 by 7.6 cm). The column is extracted with chloroform to remove adsorbed pesticides, and the extract is analyzed by gas chromatography. It is possible to obtain analyses by extraction of small volumes of water (for example, 1 liter) where concentrations of pesticides are high enough to cause a detector response at that level. Water samples containing pesticide concentrations below limits of detection are usually extracted by a countercurrent batch process or by liquid-liquid extraction processes to increase sensitivity of the method (2). Combinations of these methods have been useful for detection of extremely small amounts of contaminants but are quite time-consuming.

We have investigated the association of chlorinated hydrocarbon pesticides with microscopic particles suspended in Lake Erie. Water was collected in the vicinity of the Bass Islands in the western basin of Lake Erie from 15 feet (4.6 m) below the surface. A 5-gallon sample was centrifuged in a Sorvall RC-2B Szent-Györgyi and Blum continuous flow

centrifuge at 27,000g at a flow rate of 11 ml/min. The particulates from this fraction (0.15 $\mu$m in diameter and above) were placed on top of a preformed linear gradient of sucrose (0 to 65 percent) and centrifuged at 1500g for 60 minutes. The tube was divided into four fractions, each of which was extracted with hexane. Raw water samples (2 liters) have been extracted by using the liquid-liquid extraction procedure. No detectable chlorinated pesticides were found. Analysis of the hexane extracts for presence of chlorinated hydrocarbon pesticides was made by using an Aerograph 200 gas chromatograph equipped with an electron-capture detector [250 mc of titanium tritritide, column temperature 190°C, detector 200°C, injector port 230°C, 5-foot glass, 1/8 inch (internal diameter) column packed with Chromosorb W 60/80 mesh, coated with 5 percent Dow silicone SE-30, high purity $N_2$ carrier gas 60 ml/min].

Because gas-liquid chromatography (GLC) retention time and reinforcement of peaks are analytically inadequate for identification purposes, the presence of specific pesticides must be regarded as presumptive. The data suggested, however, that concentrations of specific pesticides associated with individual particulate fractions were below the level of detection by thin-layer chromatography (TLC) (3). Therefore, the use of confirmatory TLC on particulates from larger water samples was employed. For this purpose, a 20-gallon water sample was centrifuged as previously described. The total particulate residue was extracted with hexane and concentrated to 0.5 ml by evaporation. Aliquots (2 ul) were injected into the gas chromatograph and the remaining volume was added as a single spot onto Eastman Chromogram 6061 TLC sheets. The TLC sheets were preconditioned and developed by the methods of Kovacs (4), and sprayed with 0.05 percent Rhodamine B (3); 5 $\mu$g and 50-$\mu$g spots of control pesticides

were chromatogramed on the same TLC sheet.

The TLC sheets indicated the presence of spots with $R_F$ values identical to lindane and endrin. The GLC recordings verified the presence of lindane and endrin as well as several additional peaks from this particular sample.

Pesticides which have been tentatively identified from GLC recordings taken from particulate fractions are lindane, heptachlor, aldrin, and endrin; there were also several unidentified peaks. Pesticides were tentatively identified by comparison of retention times to those of control samples of known purity (dieldrin, aldrin, endrin, 99+ percent, Shell Chemical Co.; DDT and isomers, 99+ percent, USPHS pesticide repository; lindane, heptachlor, 99+ percent, City Chemical Corp.), and also by reinforcement of peaks by addition of known compounds. The distribution pattern of pesticides with different particulate fractions illustrates that various chlorinated hydrocarbons have an affinity for particulates separable by density-gradient centrifugation (that is, different particles). The distribution of these pesticides demonstrates their association with the particulate compounds suspended in the water. The utility of the collection and fractionation procedures is verified by the variety of pesticide separations obtained through its use.

Table 1 is a compilation of pesticide distribution and concentration in the particulate fractions of three separate lake water samples taken at different times, based upon GLC data without TLC confirmation, since the concentration of pesticide in gradient fractions is below the limit of TLC detection.

Table 1. Nanograms of chlorinated hydrocarbon pesticides per liter of lake water associated with each fraction of particulates. Samples 79 and 75 show the qualitative presence or absence of chlorinated pesticides in each extracted fraction of two other samples taken at different times of the year.

| Pesticide | Gradient fraction | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| | | *Sample 86* | | |
| Lindane | 0.53 | 4.6 | 3.2 | 16.5 |
| Heptachlor | 0.69 | 0 | 1.7 | 2.1 |
| Aldrin | 14.7 | 2.0 | 1.0 | 1.3 |
| Endrin | 9.6 | 0 | 0 | 0 |
| DDT: metabolic products or isomers | 0 | 0 | 0 | 0 |
| | | *Sample 79* | | |
| *p,p'*-DDD | + | + | | + |
| Lindane | + | | + | |
| Heptachlor | + | + | + | + |
| *p,p'*-DDT | + | | + | |
| *o,p'*-DDT | | + | | |
| Endrin | | + | | + |
| *o,p'*-DDD | | | + | |
| | | *Sample 75* | | |
| *o,p'*-DDD | + | + | + | |
| *p,p'*-DDD | + | + | + | + |
| *o,p'*-DDT | + | + | + | + |
| *p,p'*-DDT | | + | + | + |
| Lindane | | + | | + |
| Heptachlor | | + | + | + |
| Endrin | | | + | |

Evidence from this study suggests that there is a quantitative as well as qualitative distribution of pesticides associating with varied particulate components in natural waters. Pesticides that were determined were attached to particles of 0.15 $\mu$m or larger in size, and different pesticides were shown to be associated individually with particles of different density. For example, in sample 86, lindane was found in greater concentration in fraction 4, the inorganic portion of the particulate material (5), while aldrin and endrin were associated with less dense upper fractions 1, 2, and 3, which consist primarily of organics, detritus, and microorganisms. The presence of various chlorinated pesticides was associated with different particulate compounds of the environment. These compounds could be present in the environment as either molecular aggregates in aqueous solution (4.1 nm or less) or in suspension (up to 0.11 $\mu$m) (6). In our system, particulates below 0.15 $\mu$m were not included, so that the presence of pesticides is indicative of some kind of true pesticide-particulate association. The particles could be cell, detritus, or inorganic materials (for example, clay minerals).

Different chlorinated hydrocarbons are present in different water samples. For example, samples 70 and 75 suggest the presence of DDT, its isomers, and metabolic breakdown products, whereas no DDT-group compounds were detected in sample 86. The lack of detection of DDT or isomers in this sample may be a reflection of the possibility of the specific association of such compounds with specific particulates, or, of course, it may be that these compounds were below the level of detection in the quantity of particulate used for the extraction.

In analyses where activated carbon filters have been used (for example, U.S. Public Health Service Water Pollution Surveillance Program) levels (0.05 ng to 0.05 ug/liter) of pesticides are detected. This technique has made use of 30-mesh and 4-by 10-mesh carbon which has been preextracted to remove organic material. It seems likely that chlorinated hydrocarbons associated with particles that have the general size of 0.15 um could pass through coarse carbon filters and remain undetected in the water sample. Evidence from our study suggests that removal and analysis of particulates may need to be included to give more adequate estimates of pesticides in aquatic environments. In an environment such as Lake Erie, where the shallow water is so easily disturbed by wind action, the turnover and accumulation of pesticides in bottom sediments may be significant.

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Part 2.

The removal of the chlorinated hydrocarbon aldrin from lake water by flocculent bacteria.

Abstract

Floc-forming bacteria isolated from Lake Erie adsorb and concentrate aldrin from colloidal dispersion so that the settling of the bacterial flocs removes aldrin from the water phase. Contemporary sediments forming in Lake Erie contain aldrin and could adsorb more. The sediments consist of a conglomerate floc of bacteria, diatoms, and inorganic and detrital particles. Flocculent bacteria also adsorb microparticulates, and this adsorption capacity represents a mechanism for sediment formation and for the removal of suspended particles including aldrin from the water column.

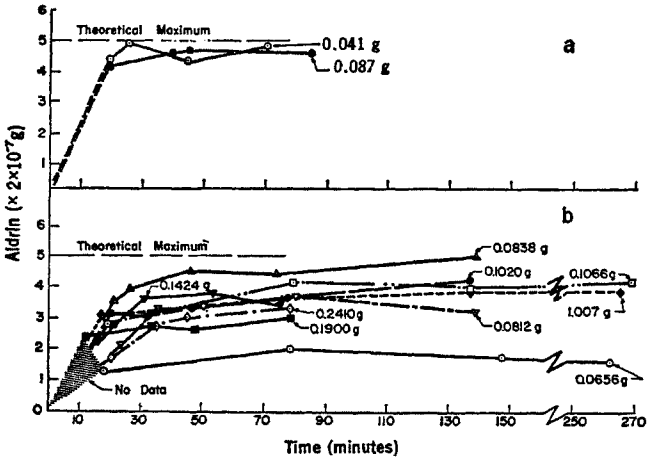
## Introduction

Many chlorinated hydrocarbon insecticides have been isolated from surface waters, usually in concentrations of less than 1 ug/liter or 1 part per billion (ppb). The deleterious effects of pesticide in water have been established (1). Our interest is in the fate of these chemicals in a water column and particularly in their adsorption to silt- and floc-forming bacteria which form contemporary sediment in lakes. Bacterial floc is an aggregation of cells which results in a macroscopic bacterial clump that settles from the liquid, thus leaving that medium less turbid. This type of growth appears to result from physical, chemical, and biological interactions when extracellular fibrillar polymers are synthesized by organisms (see 2).

Our study of aerobic bacteria isolated from Lake Erie revealed that of 33 isolates tested in six different growth media, 19 formed flocs in at least one medium, whereas ten formed flocs in two or more of the media. We report here a study of the ability of two of the floc-forming isolates to concentrate and accumulate the pesticide aldrin (3) from solution. One bacterium was an orange-red pigmented Gram-negative rod, tentatively identified as either a Flavobacterium or Protaminobacter. The other was a Gram-positive species of Bacillus.

Our experimental procedure was as follows: The test organisms were grown in a shake flask at ambient temperature ($22^{\circ} \pm 2^{\circ}$ C) in nutrient broth (8 g/liter, Difco), harvested by centrifugation, washed twice with distilled water, and resuspended in 25 ml of distilled water. Erlenmeyer flasks containing 50-ml suspensions of bacterial floc were then placed on a rotary shaker and 1 ml of aldrin dissolved in acetone was added to give a final aldrin concentration of $1 \times 10^{-6}$ g/ml or 1 part per million (ppm). After being shaken at 120 rev/min for the desired time period, the flasks were removed from the shaker and the floc was separated from the supernatant by centrifugation. The flocs were washed twice with distilled water and the washings were added to the original supernatant. The pesticide exposure time was calculated as that period between the addition of aldrin to the solution and the separation of the second washing from the bacterial floc. The floc and supernatant fractions were extracted separately with a mixture of heptane and acetone (3:1, by volume). The organic solvent fractions containing the aldrin were concentrated by evaporation and adjusted to a volume of 4 ml. Samples (2 ul each) were injected into a gas chromatograph (Aerograph model 200) fitted with an electron capture detector (4).

The total amount of aldrin adsorbed to bacterial floc as a function of time is plotted in Figure 1. The theoretical maximum for aldrin adsorption calculated from a standard curve is 1 ppm. The recovery values for aldrin varied in individual experiments between 70 and 130 percent (0.7 to 1.3 ppm), with the variation possibly due either to adsorption on glassware (5) or to the varying sensitivity of the electron capture detector. Almost all of the aldrin adsorption to floc took place




showing the inital amount of aldrin found in two different samples aldrin adsorbed during the experiment by the two samples as a

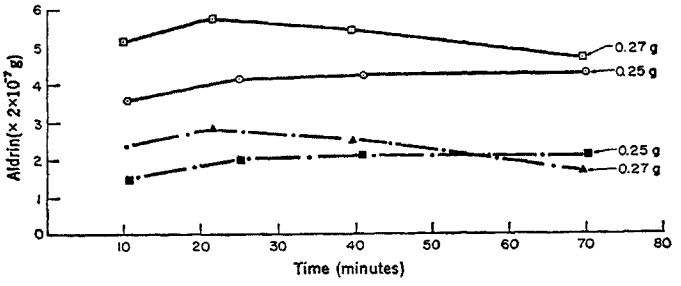



Fig. 1 (left). Curves showing adsorption of aldrin by both (a) Gram-positive bacterial floc and (b) Gram-negative bacterial floc as a function of time (solid lines). Numerals on each curve indicate the dry weight of bacterial floc used in each experiment. Broken and dashed lines indicate extrapolation from the first experimental point to zero time. Fig. 2 (right). Curves of contemporary sediment (silt) (broken lines) and the additional function of time (solid lines).

within the first 20 minutes of contact. That the amount of aldrin adsorbed in most cases remained nearly constant after 20 minutes was verified statistically by single variable linear regression at both the 1 and the 5 percent levels of significance when the amounts of aldrin adsorbed as a function of time were compared. All of the aldrin added to the Gram-positive bacteria was recovered from the floc; none was recovered from the supernatant. The recovery value averaged 88 percent. The slope of the line is linear and $\beta = 0$ (6) at both the 1 and 5 percent significance levels when floc weight as a function of pesticide adsorbed was evaluated. This linearity is due to the high rate of aldrin adsorption by floc which resulted in maximum adsorption within the minimum time period required to obtain the first adsorption value (that is, 12 to 15 minutes). The fact that there was no significant difference in the amount of aldrin adsorbed by 0.041 g of floc as compared to 0.087 g of floc indicates that 0.41 g was sufficient to adsorb all of the available aldrin.

Adsorption curves shown in Figure 1 indicate a rapid uptake of aldrin during the first 20 minutes until maximum theoretical adsorption is reached. As aldrin is adsorbed, less is available in solution to be adsorbed. Therefore, the flocs effectively adsorb from more dilute solutions than anticipated by the initial test concentration. This would essentially represent adsorption from lower, more realistic, pesticide concentrations found in Lake Erie.

Data for the Gram-negative organism show that the amount of aldrin adsorbed by an equal weight of cell flocs remained the same or increased only slightly with time beyond 20 minutes and either decreased or remained unchanged in the supernatant. The adsorption curve was linear at the 1 and 5 percent significance levels, but only if the 0.0656 g value was omitted from the calculations. If this value was included, the data did not represent a linear relation at either level. However, in neither case was $\beta = 0$, an indication of a relationship between adsorption and floc weight in this case. Deviation from $\beta = 0$ results from a slower adsorption by the Gram-negative bacteria as compared to the Gram-positive bacteria.

The concentrating effect of these bacteria is considerable. For example, when 0.041 g of Gram-positive floc adsorbed pesticide from 25 g of water, the concentration factor was about 625 to 1 within 20 minutes. A similar but slightly smaller amount of adsorption occurred with the Gram-negative organism. Analogous findings have been reported for algae (7).

Samples of natural sediment that were in the process of settling and accumulating in Lake Erie were collected by specially designed sediment collectors placed on reefs (8). This sediment consisted primarily of inorganic matter. We analyzed the sediment in a matter identical to that described for bacterial floc, and we also examined the sediment on a microcoulometer (Dohrmann Instrument Company model 200A) (9).

The presence of both aldrin and dieldrin (3) in contemporary sediment was detected by both gas chromatography and microcoulometry. Additional aldrin added experimentally was absorbed and, as shown in Figure 2, the concentrations after 10 and 70 minutes were almost equal. No aldrin was detected in the supernatant. The data were linear at both the 1 and 5 percent levels of significance and was equal to zero.

Contemporary lake sediments appear to accumulate pesticide from suspension in a manner similar to that shown for bacterial floc. Floc-forming bacteria are common in the lake environment and experimentally have a rapid and high adsorption capacity for aldrin. Organic "floc-like" bottom sediments from the eastern basin of Lake Erie have been reported (10), and electron microscopic examination of contemporary sediments from Lake Erie shows that these sediments consist of a conglomerate of bacteria, diatoms, and inorganic and detrital particulates.

Counts of $10^5$ aerobic and $10^6$ anaerobic heterotrophic bacteria have been obtained per gram (wet weight) of contemporary lake sediment. In this regard, clay particles are known to adsorb pesticide and lake sediments are known to adsorb lindane (3,11). Pfister, et al, (12) reported that chlorinated hydrocarbons both behave as suspended micro-particulates and are associated with other microparticulates including detritus in the water column. Other researchers (13) have established that DDT (3) is taken up from organic detritus by fiddler crabs. Significant concentrations of chlorinated pesticides have been detected in algae and lake bottom mud (14). It is known that actinomycetes, fungi, and other bacteria adsorb and concentrate pesticides from solution (15),

and that microparticulates associate with microorganisms (16). Particulate organic material has been considered a potentially important source of food for filter-feeding marine organisms (17). The suggestion has been made that most of the particulate organic carbon at depths shallower than 175 m in the Atlantic Ocean off South America consists of living organisms and decomposable organic matter (18).

We conclude that floc-florming microorganisms act as adsorbants for other suspended microparticles including chlorinated hydrocarbons and that this adsorption represents a natural process for the removal of microparticles from the water column. Once the microparticles have settled from suspension, the fate of the pesticides is in question, but they may be degraded under anaerobic conditions (19). It is likely that pesticides concentrated in bottom sediments for even short periods of time would exert an insecticidal effect on the bottom insects and other susceptible fauna. Jensen and Gaufin (20) and Carlson (21) have shown that different species of stone fly and mayfly naiads have varying susceptibilities to the same pesticide. This may explain the disappearance of certain insects from Lake Erie such as mayflies, and the persistence or increase of others. The same may hold true for other organisms in the lake.

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4. Operating conditions were as follows: 250 mc of titanium tritritide, $Ti^3H_3$; column temperature, 185°C; detector temperature, 200°C; injector port temperature, 225°C; glass column 152 cm long and 0.32 cm in internal diameter packed with Chromosorb W 60/80 mesh, coated with 5 percent Dow silicone SE-30; high-purity nitrogen carrier gas; flow rate, 60 ml/min.

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9. Inlet temperature, 300°C; furnace temperature, 800°C; equipped with a glass column 182 cm long and 5 mm in internal diameter, packed with Chromosorb W 60/80 mesh, coated with 7.55 percent QF-1 and 5.20 percent DC 200; high-purity nitrogen gas; flow rate, 190 ml/min.

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22. Supported by grant No. B-013 from the Office of Water Resources Research, and grant No. WP-00713 from the Federal Water Quality Administration, U. S. Department of Interior. We thank E. Herdendorf of the Ohio Department of Natural Resources Geological Survey for supplying the contemporary sediment from Lake Erie.

Part 3.

Particulate fractions in water and the relationship to aquatic microflora.

Abstract

This investigation was to study the interaction of environmental contaminants (defined as substances not formed biologically or naturally and which are not normally indigenous to the water) on the microbial portion of the ecosystem. Particulate suspended materials (minerals and detritus) were examined on a physical and biological basis, and characterized using differential and gradient centrifugation in conjunction with electron microscopy. Several characteristic fractions of suspended particulate material were examined for ability to influence biological reactions. The particulate fraction of water is important to microbial relationships in the area of interfaces and biological activity. It is known that particles and molecules in solution accumulate at interfaces (this includes chemicals which can either act favorably (nutrients) to organisms or unfavorably (pesticides) to organisms), and that enzymatic reactions are concentrated at membranous surfaces. Therefore, it is of significant importance to study the capabilities of non-biologicals that commonly end up in the waters on such colloidal or molecular interfacial systems.

## Introduction

The natural process of eutrophication appears to be greatly accelerated in Lake Erie. The net effect of accelerated eutrophication involves stimulated shifts in predominant organisms, changes in growth patterns, and an overall biomass increase in the Lake. This could result from a variety of contributary factors; e.g. cities, agriculture, erosion, industrial wastes, heat from power plants, etc.

At the physiological level, growth of organisms can be interpreted in physical-chemical and nutritional terms. The processes then would be related to the extent and nature of growth promoting, or inhibiting, substances which enter the Lake; and therefore to streams in the watershed that carry material into the Lake. Many of the substances that enter the Lake are wastes which can be modified, via the metabolic activities of protists and other microorganisms, to serve as nutrients for higher life forms. This includes the conversion of such compounds as detergents, hydrocarbons, cellulose, chitin, etc. to microbial cell mass which can then be utilized directly by higher forms.

Metabolic activities, hence total growth, of microbes tend to increase at interfaces (Bigger, 1941; ZoBell, 1943; Zvyagintsev, 1962). Indeed, the reason for extremely high metabolic rates in protists, as compared to that in multicellular organisms, is largely due to their high surface to volume ratio. Microorganisms are a system of surfaces composed of membranous organelles.

It is known that suspended particulates as well as dissolved chemicals tend to accumulate at interfaces (Henrici, 1935; Heukelekian, 1940; Riley, 1963; Wood, 1962). This phenomenon is the basis of foam or froth flotation recovery processes. Further, suspended particulates, when available, represent active sites for adsorption and concentration of dissolved chemicals at the surface. This is the physio-chemical basis for many types of chromatography and purification processes.

The purpose of this investigation is to examine the interaction of suspended particles and growth of microorganisms in relation to accumulation of dissolved nutrients in the Lake, with an ultimate objective of determining whether or not these processes have a significant influence on eutrophication. Particulates in the size range of 10 microns or less have been chosen for study because the high surface to volume ratio is likely to be quite significant in relation to accelerated growth rates.

## Methods and Results

Figure 1 shows a flow diagram for the analysis of water. Water samples were collected from a depth of 15 ft in the Lake, or just below surface in the Sandusky River. The diagram illustrates that centrifugation was used in the process. This was done by collecting a 5-10 gallon sample. The bacterial counts and initial isolations of bacteria were done as soon as possible upon retrieval of the water sample and were frequently accomplished (weather permitting) on board the boat. Incubation was carried out at 30°C.

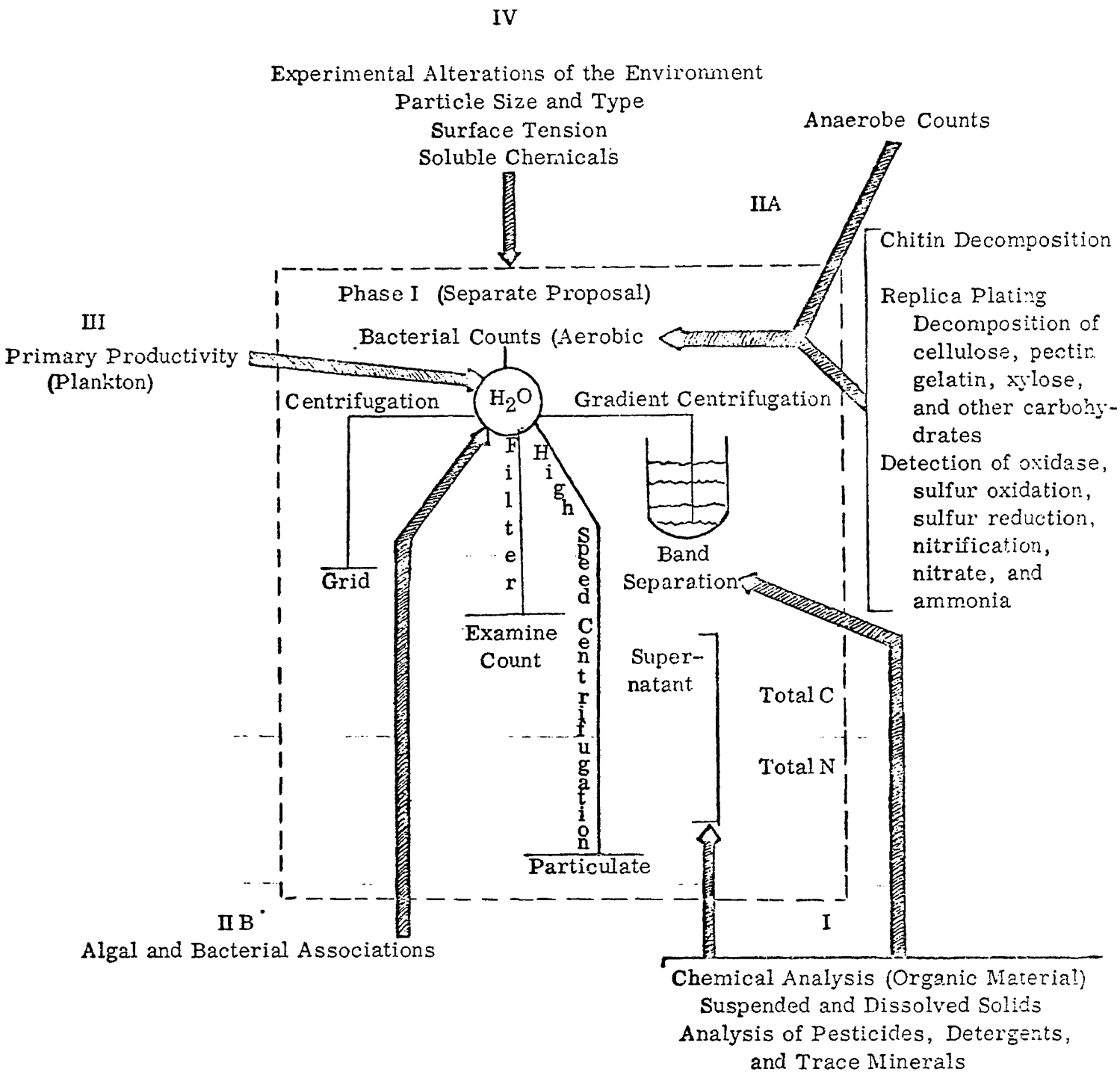


Figure 1. The flow diagram within the box indicates experiments conducted as Phase I, a separate allotment project. The evaluations indicated outside the box represent an extension and utility of the initial project data and represent research which was the basis of this proposal.

Water was centrifuged at 27,000 x g with a flow rate of 45 ml/min in a Sorvall Szent-Gorgi continuous-flow centrifuge. This permitted the removal of particles down to 0.3 microns. The supernatant water was then passed through the centrifuge at 27,000 x g with a flow rate of 11 ml/min which removed colloids down to 0.1 micron. Solid residues from these fractionations were weighed and placed on top of a gradient constructed of sucrose with a linear density of 1.0765 to 1.2241 and centrifuged at 1,500 x g for 60 min (Lammers, 1962, 1964 and 1967).

Bands were collected by using a Beckman tube-cutting device and particulates in each band were either dialysed against distilled water or washed in distilled water by high-speed centrifugation. 0.01 ml of each fraction was placed on a carbon-coated electron microscope grid, dried and examined. Both inorganic and organic particles could be examined and estimated as to size, distribution and density (Figure 2). It can be seen that the inorganic particles, including diatoms, are found at lesser densities. Particles in this treatment will separate primarily on the basis of density.

Another method which we have employed to separate particulates on the basis of size is the direct preparation of carbon replicas on membrane filter surfaces (Millipore Corp., Bedford, Mass.).

Volumes of from 5 to 50 ml of water were pushed through the filter before it was removed and air dried. 1 mm square pieces were cut from the sample, shadowed and carbon replicated. Carbon films were washed in acetone, chromic acid and water. Figure 3 shows an electron micrograph of the surface of a standard unused 0.45 micron pore size filter.

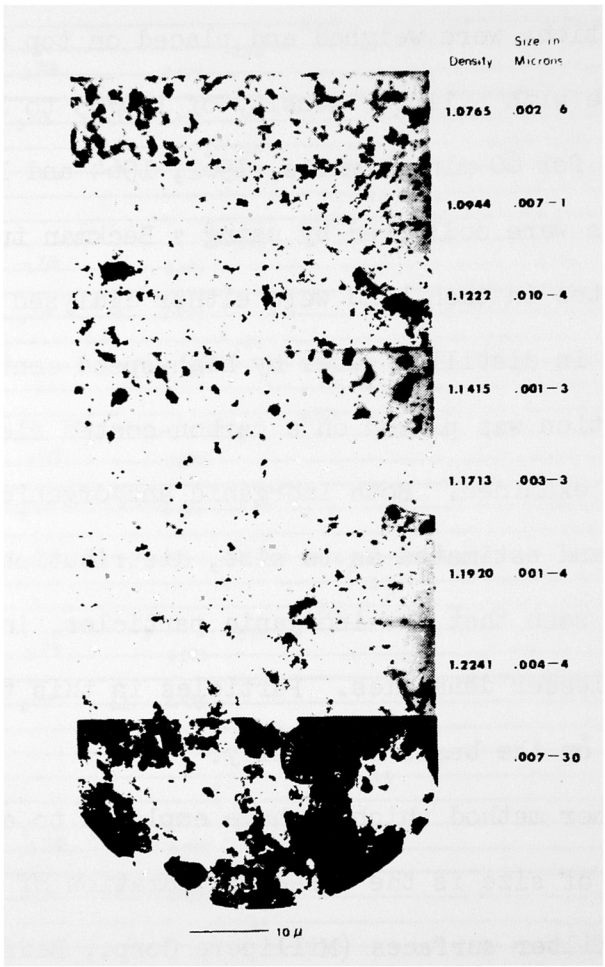


FIGURE 2. COMPOSITE PHOTOGRAPH MADE FROM THE EXAMINATION OF PARTICULATE FRACTIONS SEPARATED BY USING A LINEAR SUCROSE GRADIENT. EACH SEGMENT IN THE PHOTOGRAPH REPRESENTS A SECTION IN THE GRADIENT REMOVED WITH A TUBE-CUTTING DEVICE.

3

FIGURE 3. ELECTRON MICROGRAPH OF A CARBON REPLICA OF A STANDARD UNUSED .45 MICRON PORE SIZE MILLIPORE FILTER.

In Figure 4 a variety of particulates can be seen on the surface. Chromic acid in the washing process is not particularly effective against inorganics, and these can be seen as dense electron-scattering particles. The other particles, which have been removed by the treatment and are seen as the same density as the background carbon, were probably organic in origin.

To examine the biological effects of particulates which had been fractionated as described, several experiments were attempted, Table 1. Pure cultures growing on half-strength tryptone glucose yeast extract broth were subjected to the addition of particulate fractions and their supernatents. The histogram shows the growth of each culture as compared to a control designated as "no fraction" in the graph. Each bar of the histogram has been corrected for effects of turbidity caused by the addition of the particulates.

Results show thatin three of the organisms examined, the particles 0.3 micron or larger were effective in inhibiting the growth as measured turbidimetrically. The growth of the yeast culture appeared to be slightly enhanced by the particulates. The explanation of these effects has not been determined.

Another experiment was conducted with pure cultures of Micromonospora sp. and Streptomyces sp. which had been isolated from Lake Erie. The particulate fraction 0.3 micron and larger was added to a salts medium which contained no carbon source. The addition of these particulates stimulated a significant increase in relative biomass over the control cultures which recieved no particulates. The arrow indicates

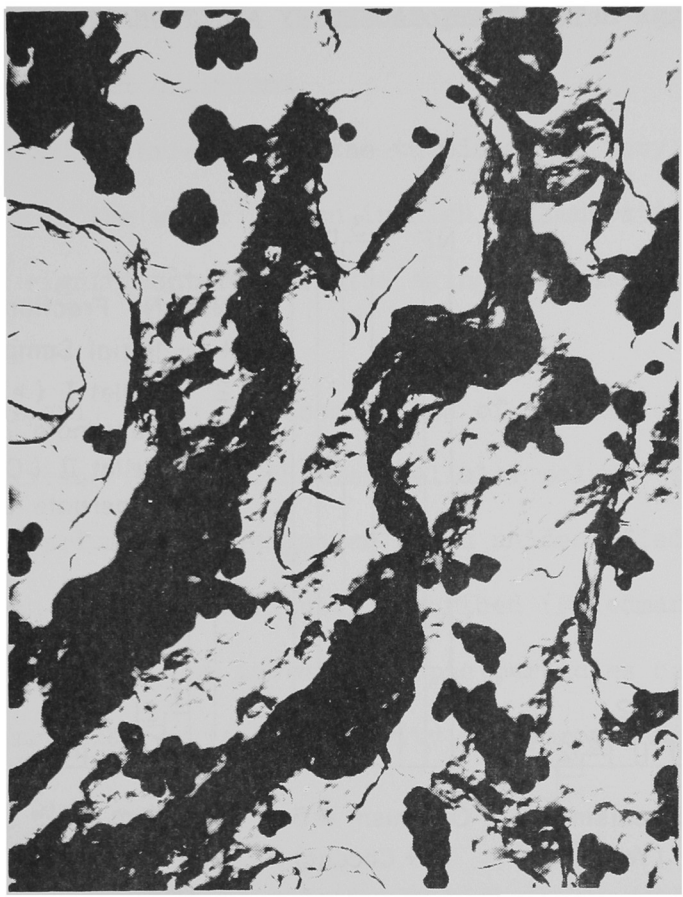

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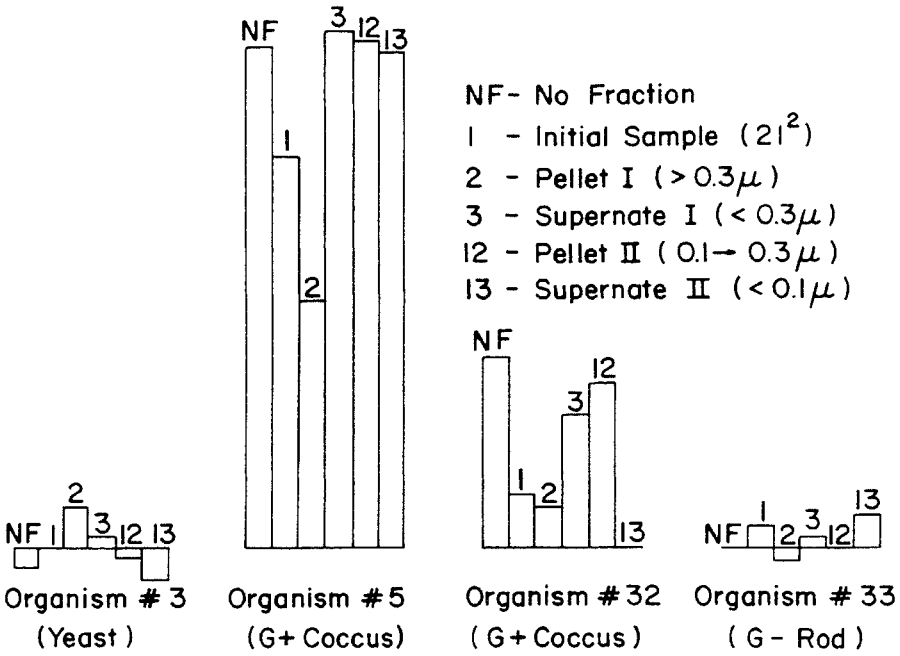
FIGURE 4. CARBON REPLICA OF A MILLIPORE FILTER AFTER FILTERING 10 ml OF LAKE ERIE WATER. DARK PARTICLES ARE PROBABLY INORGANIC.
30,000X

TABLE 1. RESULTS OF AN EXPERIMENT SHOWING THE EFFECT ON MICROBIAL GROWTH BY TWO PARTICULATE FRACTIONS AND THEIR SUPERNATENTS. THE BARS OF THE HISTOGRAM REPRESENT THE RELATIVE GROWTH MEASURED TURBIDIMETRICALLY AS COMPARED TO A GROWTH CONTROL (NO FRACTION). EACH BAR HAS BEEN CORRECTED FOR THE EFFECTS OF TURBIDITY CAUSED BY ADDITION OF THE PARTICULATES.



the concentration of this particulate fraction in the Lake. Results of this experiment are shown in Table 2.

Figure 5 shows the Streptomyces attached to particles in this experiment. This appears to be the development of an aggregate between suspended particles and organisms.

In a lake such as Erie, where the turbidity is very high and particulates are in the water column in high concentration, they probably play a significant role in the aging and eutrophication of the lake.

Figure 6 clearly shows the association of sub-microscopic particles of magnesium silicate (talc) with an exocellular polymer produced by this aquatic bacterium. This organism is an unidentified floc-forming pseudomonad which has been previously described (Friedman, 1968). This serve to illustrate how sub-microscopic particles can be aggregated into a larger particulate via the activity of microorganisms.

## Discussion

The water in Lake Erie is relatively high in suspended particulates and this may be related to reported increases in the rate of eutrophication.

This report indicates that the particulates in the Lake are comprised of substances having a variety of densities and that fractions can be separated which exert an influence on the growth and metabolism of microbes taken from the Lake. It can therefore be concluded that specific aquatic microbes have the capacity for accumulating inorganic

TABLE 2. RESULTS OF AN EXPERIMENT SHOWING THE EFFECTS OF THE ADDITION OF A 0.3 MICRON AND LARGER PARTICULATE FRACTION ON THE GROWTH OF STREPTOMYCES SP. AND MICROMONOSPORA SP. THE GRAPH SHOWS INCREASE IN RELATIVE BIOMASS AS MEASURED BY INCREASE IN DNA CONTENT THROUGHOUT THE EXPERIMENT. THE ARROW INDICATES THE CONCENTRATION OF THIS FRACTION IN THE LAKE.

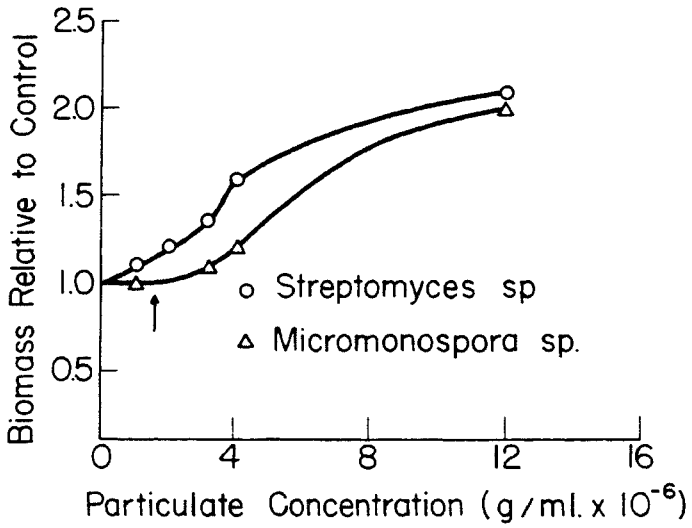


FIGURE 5. STREPTOMYCES SP. ISOLATED FROM LAKE ERIE SHOWN ASSOCIATING WITH 0.3 MICRON AND LARGER PARTICULATE FRACTION FROM LAKE ERIE. THE PARTICLES IN THIS EXPERIMENT ARE THE SOLE SOURCE OF CARBON. 1,600 X

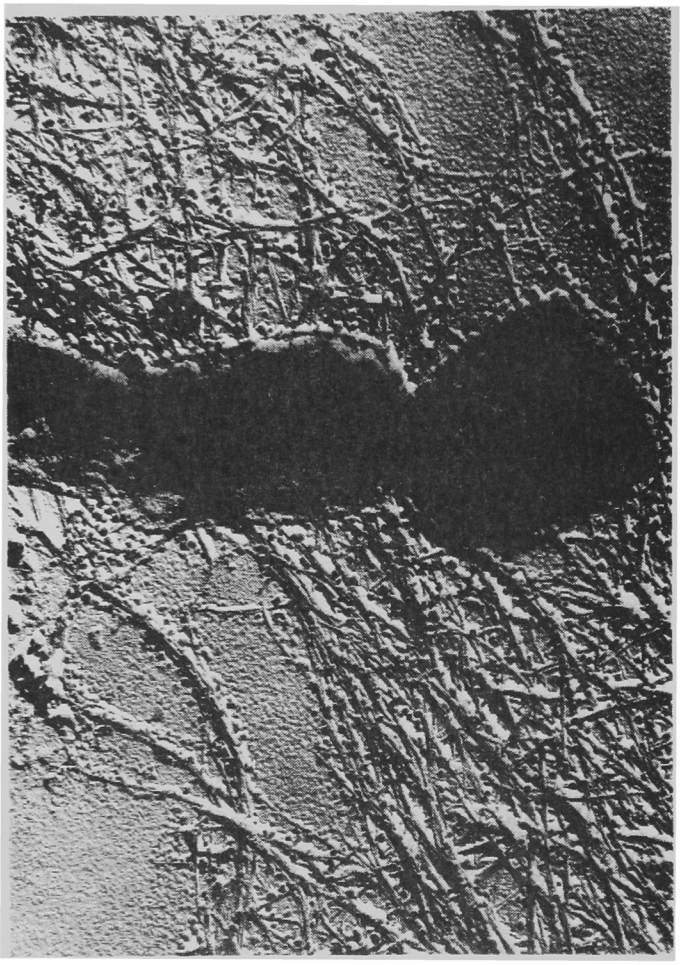


FIGURE 6. THE PHOTOGRAPH SHOWS THE ASSOCIATION OF MAGNESIUM SILICATE (TALC) WITH A POLYMER PRODUCED BY AN UNIDENTIFIED FLOC-FORMING PSEUDOMONAD. 25,000 X

micro-particulates. This in effect alters the distribution and availability of micro-particulate surfaces and may even remove them from suspension via a flocculation process. Growth of the organisms to which micro-particulates become attached is known to be controlled by available nutrients.

Postulation of a system of ecological controls can be made which involves interaction of nutrient concentration, suspended micro-particulates, and buildup of larger aggregates from association of inorganic and living particles.

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Part 4.

The effects of microparticulates and chlorinated hydrocarbons on microorganisms isolated from Lake Erie.

Abstract

Water samples from the western basin of Lake Erie have been analyzed with regard to the distribution of colloidal microparticles. Size analyses of particulate samples placed on a sucrose density gradient revealed that the most common size particle was in the range of 0.1 um. Chlorinated hydrocarbon pesticides such as endrin, aldrin, heptachlor and lindane were found in association with these particles and the data suggest that aldrin and heptachlor were found more frequently on the smaller, less dense particles, while lindane was associated with the larger, more dense fractions. Bacteria isolated from these water samples prior to chemical analyses were grown in the presence of clay microparticles freed of pesticides, microparticles containing known amounts of pesticides, and purified pesticides alone. Bacterial growth effects were measured by changes in the turbidity of the medium, total DNA content of the culture and standard plate counts. Results demonstrate that different bacteria in the presence of endrin or aldrin could be affected in different ways. In some cases the organisms were stimulated to produce a cell yield of 4-5 times that of the control cultures. A survey of 151 heterotrophic aerobic bacteria isolated from Lake Erie has shown that 55 were stimulated by aldrin, 54 by endrin and 45 by dieldrin. Forty-six cultures were inhibited by aldrin, 43 by endrin and 43 by dieldrin. Eighteen cultures were stimulated by the three compounds, while 27 cultures were inhibited.

## Introduction

The presence and persistance of chlorinated hydrocarbon compounds in the aquatic environment has presented an interesting and potentially dangerous ecological situation. The wide distribution of these recalcitrant organic molecules has raised the question of their long-term effects on our natural environment. The occurrence of these compounds as either molecular aggregates in aqueous solution (4.1 nm or less) or in suspension (up to 0.11 μm) (Bowman, et al., 1960) in the water and the association of these compounds with particulate suspended solids is significant. The interactions and metabolic activites (e.g. growth) of organisms tend to increase at interfaces (Bigger, 1941; ZoBell, 1943; Zvyagintsev, 1962; Pfister, et al., 1968).

It is also known that suspended particles and dissolved chemicals accumulate at interfaces (Riley, 1963; Wood and Oppenheimer, 1962) and that when present represent activite sites for adsorption and concentration of materials at the surface. The presence and distribution of pesticides as particulates or in combination with other micro-particulates through adsorption or as stored products in or on minute organisms such as bacteria must be carefully examined and understood. The response of the environment to these compounds must be learned in order to predict or directcorrective measures to be taken to insure environmental safety. Lake Erie is an ideal lake for these studies because of its high rate of man-made organic pollution and its high particulate content.

The purpose of this investigation was to analyze water samples taken from the western basin of Lake Erie (Figure 1) with respect to colloidal particle distribution and size, pesticide content and distribution in the particulate fractions and effects of certain clay and pesticide particulates on bacterial growth.

## Methods and Results

Water samples were collected from a depth of 15 feet in Lake Erie in 5 gallon quantities. Heterotrophic bacteria were plated out immediately on plate count agar (PCA). These platings were done as soon as possible upon retrieval of the water sample usually on board the boat. Incubation was carried out at $25^{0}$C. After 48 hours, single colonies were picked and restreaked for further experimentation.

The five gallon water sample was passed through a continuous flow high speed centrifuge (Sorvall RC-2B equipped with a Szent-Gorgi continuous flow attachment) at a flow rate of 11 ml per minute and a gravitational force of 27,000. Solid residues from the water sample were weighed and placed on top of a linear gradient of sucrose with a density from 1.0765 to 1.2241. The preparation was centrifuged at 1,500 x G for one hour (Lammers, 1962, 1964, 1967).

Five bands were collected using the tube cutting technique and the microparticles in the bands dialysed against or washed in distilled water by high speed centrifugation (27,000 x G for 30 min.). When

pesticide analysis was to be carried out, the gradient was divided into four bands which were each separately extracted in hexane. The analysis of the hexane extracts for the presence of chlorinated hydrocarbon pesticides was made using an Aerograph 200 gas chromatograph (GLC) equipped with an electron-capture detector (Pfister, Dugan and Frea, 1969).

Particulate Matter in Lake Erie Water Column

Particulates from Lake Erie water samples were evaluated for individual size and size distribution using the Particle Size Analyzer TGZ-3 (Zeiss Co.). This instrument utilizes an illuminated iris diaphragm which can be varied in diameter so that its area can be made equal to the area of a photographed particle (Falcon-Uff, Leverington, 1967).

Lake Erie samples (111A, 111B, 115, 117) were used in this analysis. Samples were prepared for photographing by placing 1-3 micrograms of the particulates on a carbon-coated electron microscope grid. The grids were observed in an Hitachi (HS-7) electron microscope. The fields photographed were both representative of the sample and contained particles which were sufficiently dispersed so that individual particles could be identified (i.e., as little overlapping as possible). On the average, each field contained 100-200 countable particles. Photographic negatives were enlarged so that the smallest particles were not less than 1-2 mm and the largest did not exceed 37.7 mm. Total magnification remained constant throughout a fraction which was to be sized. Photographic positives were made using Kodak Ektamatic paper.

Sizing was accomplished using the exponential-distribution-standard operating condition of the instrument. Each particle was analyzed by placing its photograph over the iris diaphragm, and adjusting the diameter of the circular light spot to equal the area of the particle. If a particle deviated from a circular shape, the light spot was adjusted so that the total area of the protruding portions of the particle became equal to that of the re-extrant areas. The particle was then recorded in a specific counter category in the instrument. A minimum of 1,000 particles per fraction was counted.

Interval centers of the counters were then converted to the actual size of the particles by dividing them by the total magnification. The readings (numbers of particles) obtained on the individual counters were multiplied by the correction factor for exponential counting. The corrected interval centers and the corrected number of particles were then plotted to give a distribution curve. Distribution curves were done on all fractions within a sample (Figure 3-7), and a total distribution curve was plotted for the entire sample (Figure 2).

A student-T test for statistical fitness was also done comparing the samples. The average particle size ranged between 0.14 and 0.24 $\mu$. The absolute particle range, however, was as low as .029 $\mu$ and as high as 7.90 $\mu$ (Table 2).

In the individual fractions a large peak in the number of particles usually occurred in the 0.08 to 0.2 $\mu$ range. This range accounted for, on the average, 50-55% of the total particles counted.

The T-statistic information (Table 3) shows that all three samples were significantly different.

Pesticide Distribution on Microparticulates

Examination of particulates from five water samples for the presence of chlorinated hydrocarbons showed that there was a distribution of these compounds through the gradient. In these five cases, the gradient was divided into four sections, each of which was analyzed separately. With only five complete samples, no real significance could be attached to the distribution patterns with respect to each original water sample. When the total amount (nanograms) of each pesticide was calculated and an indication of the fraction from which it was isolated was made, a distribution pattern emerges. Figure 8 shows the chlorinated hydrocarbons dieldrin; p,p'-DDE; endrin; heptachlor; aldrin; lindane; and o,p'-DDD and their cumulative distribution (from five samples) in the four fractions from the sucrose gradient. In the case of endrin and aldrin, the largest amounts were recovered from fractions 1 and 3 when compared to fractions 2 and 4. Very little lindane was recovered in fractions 1 and 2 (about 125 nanograms) while 680 nanograms were recovered in fractions 3 and 4. The o,p'-DDD appeared to be more evenly divided with slightly more in fraction 4. Dieldrin and p,p'-DDD were also more evenly divided in all fractions except for fraction 3. The lowered amount in fraction 3 could also be seen in the o,p'-DDD and the heptachlor samples where most of the pesticide remained in fractions 1 and 2.

Interaction of Pesticides and Microparticles on Bacterial Growth

In this study we have exposed a culture of a Pseudomonas species isolated from Lake Erie (OSU isolate #501) as previously described (Pfister, Dugan and Frea, 1968) to various concentrations of the chlorinated hydrocarbon pesticides endrin and p, p'-DDE. These compounds were placed in suspension using double distilled hexane or were adsorbed to the clay kaolinite after it had been preextracted and washed in hexane and acetone. In order to adsorb pesticides onto the clay, 0.3 g of kaolinite was suspended in 100 ml double-distilled demineralized water. One ml of a stock solution (endrin, 0.1 g/ml or 0.001 mg/ml; p,p'-DDE, 0.01 mg/ml or 0.001 mg/ml) of pesticide was added and left on a rotary shaker for 24 hours at 21°C. The clay was then washed three times in double-distilled, demineralized water to remove any unadsorbed pesticide aggregates. Samples of the extracts and the presence of the hydrocarbons on the clay were examined using the GLC technique. The final concentration of endrin in the medium was 3.7 $\mu$g/ml (conc) and 3.7 x $10^{-4}$ $\mu$g/ml (dil) of p,p'-DDE was 3.7 x $10^{-3}$ $\mu$g/ml (conc) and 3.7 x $10^{-4}$ $\mu$g/ml. The bacteria were cultured in a defined medium as shown in Table 1. All cultures were grown on a rotary shaker at 21°C.

Growth was determined through measurement of turbidity (Klett-Summerson Colorimeter equipped with a 540 NM filter) every24 hours and with a diphenylamine assay for analyses of total deoxyribose nucleic acid (DNA) at completion of the experiment. (Dische, 1955). All experiments were repeated in triplicate.

In order to relate DNA content to dry cell weight, cells were washed and suspended in distilled water and measured amounts pipetted into tared weighing cups. An equal amount was put in a glass centrifuge tube and the DNA content determined with diphenylamine. The weighing cups were dried in an oven at 100°C and weighed at 24 hour intervals until the weight remained constant. DNA content and dry cell weight were then plotted relative to an untreated control (Figs. 9,10).

In order to relate the increase in turbidity observed during the experiments and the increase in DNA content with an increase in cell number, viable plate counts were also made. These were done every 24 hours by dilution in sterile water blanks followed by plating on PCA (Difco). Plates were incubated for 48 hours at 21°C and counted at the appropriate dilution. Results of these counts always showed a consistent increase in viable cells which followed substantially the increases in turbidity and DNA.

The growth rate of a Pseudomonas sp. in defined medium was found to be greatly enhanced by the addition of 0.04 grams of kaolinite per 35 milliliters. With the addition of 0.13 ml double-distilled hexane, an increase in growth was also observed. In the case where pesticides were added in a non-adsorbed aggregated form, this acted as a control since the pesticides were dissolved in hexane. A sample of each pesticide was adsorbed on kaolinite and the effect tested on growth. Concentrated p,p'-DDE enhanced growth both by itself and while adsorbed to the kaolinite. Dilute p,p'-DDE enhanced growth slightly while adsorbed to clay, but caused a decrease in growth when by itself. The amounts of growth measured by turbidity and DNA in each experiment were closely parallel throughout

the experiment (Figure 9). Concentrated endrin seemed to inhibit growth while dilute endrin caused an increase (Figure 10).

Survey of Lake Erie Heterotrophic Bacteria in Response to Pesticides

A survey of the response of 151 heterotrophic aerobic bacteria isolated over a two year period from Lake Erie to the presence of three chlorinated hydrocarbon pesticides has been carried out. Aldrin, endrin and dieldrin were placed in 9.9 ml of the medium previously described to a final concentration of 1.0 $\mu$g/ml. Acetone was used to dissolve the hydrocarbons and 0.1 ml of the stock solution was added to each growth flask. Incubation was carried out at $25^{\circ} \pm 2^{\circ}$ for one week in quiescent culture. Five growth flasks in replicates were prepared and samples monitored for absorbancy compared to control flasks in a Coleman autoset spectrophotometer. The results of these analyses indicated that many of these cultures were changed in their growth patterns and were in some cases significantly stimulated or depressed in the presence of these compounds. Of the 151 cultures examined, 55 were stimulated by aldrin; 54 by endrin and 45 by dieldrin. Examination of the results showed that 46 cultures were inhibited by aldrin; 43 by endrin and 43 by dieldrin. In many instances, cultures were effected by more than one pesticide. All three pesticides were stimulatory to 18 of the cultures, while 27 were inhibited by them.

Discussion

These data show (Figure 3-7) that in the sample tested from Lake Erie (Zone A, Figure 1) there was a distinct distribution of micro-particles which could be separated by density gradient centrifugation. Figure 2 shows the population of particles from sample 111B prior to having been centrifuged in the sucrose gradient. The greatest number of particles are found in the size range of 0.05-0.2 microns (colloidal size). This has been the case in every sample examined to date and can be seen in the results shown in Table 2. In sample 111A taken on 3/21/69 in Zone A, there was an average particle size of 0.24 microns with an absolute range of 0.029 to 7.9 microns. Each of the other samples 111B, 115 and 117 reflect essentially the same result which suggests that the majority of particles are indeed in the small size range.

Additionally, it can be seen that the particles from Zone A and Zone B taken on the same date only one hour apart have significantly different size (Table 3). The particles from Zone B, which is east of Zone A are smaller in average size. Lake Erie is known to become less turbid toward the eastern end, so it is not surprising to find smaller particles in Zone B. When samples were taken in Zone A or B on different dates, under different weather conditions (e.g.,wind direction, velocity, and temperature differences) the particle sizes (sample 115,117) were also different (Table 3). Since Lake Erie is shallow, especially in the western basin, altered wind direction and velocity severely affects the particulate content of the water. It can be generally concluded from these data that the majority of the particles are colloidal in size and

that they do vary with either location or time of sampling. It will be of future interest to learn the associations or interactions of microorganisms with the varying particle content. This is of importance because the particles can be separated in sucrose gradients (Figures 3-7) and have been found to demonstrate a varied chemistry. In this study (figure 8) we have concluded that different hydrocarbon pesticides may be adsorbed or absorbed on or in different particles. Whether or not this is particle-specific is not presently known, but the data suggests that certain compounds can be found in association with lighter, smaller or more dense or larger particles. This finding is interesting in light of the fact that different particle sizes may be found in the lake as previously discussed. It is obvious then, that the consideration of not only the weather, but the type of particle input into the lake becomes significant with respect to involvement in the microecology.

The presence of recalcitrant organic pollutants is increasing in the natural environment and it has been generally conceded that the problems are arising because of the inability of microorganisms to degrade these pesticides, at least not at rates which prevent accumulation and serious water or soil pollution. Examination of Figures 9 and 10 raise still another concern as yet unexplored. Microparticles isolated from Lake Erie do effect the growth of the cultures isolated from the lake. The experiment shown here (Figure 9) demonstrates that p,p'-DDE in low concentration does stimulate a bacterial culture. The clay microparticles themselves stimulated the growth of this culture, and the combination

of microparticles containing pesticide was most satisfactory in enhancing growth. The compound endrin and the same culture had less pronounced effects, but even there, stimulation of the cells by the washed clay and the pesticide adsorbed clay was detected.

The association of these chlorinated hydrocarbon chemicals with certain components of the colloidal particle system in Lake Erie appears to be a significant one and deserves future study. Whether or not these effects are widespread in the microecology also needs to be evaluated. We have examined 151 heterotrophic bacteria isolated over a 2 year period in the western basin of Lake Erie. The results of this study suggest that a large percentage of these types are affected by the presence of such recalcitrant molecules whether they utilize the compounds in some way or not. The important point is that they may have an effect on the microbial microenvironment and must be considered as potentially dangerous long-term interlopers. As pesticide-laden sediments accumulate in a lake such as Erie, and it becomes more eutrophic, these effects will become more prominent. How these reactions effect the primary productivity of any body of water is of prime importance.

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TABLE 1

| | g/l |
|---|---|
| arginine | 1.0 |
| dextrose | 10.0 |
| $MgSO_4 \cdot 7H_2O$ | 0.4 |
| $K_2HPO_4$ | 4.0 |
| $KH_2PO_4$ | 2.0 |
| xanthine | 0.005 |
| guanine | 0.005 |
| adenine | 0.005 |
| uracil | 0.005 |
| riboflavin | 0.005 |
| nicotinic acid | 0.025 |
| B-alanine | 0.025 |
| Pyridoxine | 0.010 |
| Folic Acid | 0.00005 |
| Biotin | 0.0001 |
| PABA | 0.005 |
| Vitamin $B_{12}$ | 0.000003 |

TABLE 2

| Sample | Date | Location | Corrected No. Particles Counted | Average Particle Size | Absolute Particle Range |
|---|---|---|---|---|---|
| 111-A | 3/21/69 | Zone A | 21,070 | 0.24 u | .029 u to 7.9 u |
| 111-B | 3/21/69 | Zone B | 11,252 | 0.14 u | .039 u to 1.5 u |
| 115 | 5/16/69 | Zone B | 9,433 | 0.22 u | .061 u to 2.60 u |
| 117 | 5/29/69 | Zone A | 6,033.9 | 0.19 u | 0.062 u to 2.60 u |

TABLE 3

| Sample | Sample | T-Statistic |
|---|---|---|
| 111-A | 111-B | 29.60 |
| 111-A | 115 | 5.15 |
| 111-A | 117 | 10.35 |
| 111-B | 115 | 39.20 |
| 111-B | 117 | 29.86 |
| 115 | 117 | 10.36 |

# FIGURE LEGENDS

Figure 1. Map of western basin of Lake Erie showing areas of study (Zone A and Zone B).

Figure 2. Total size distribution of microparticles in sample 111B before gradient separation.

Figure 3. Fraction of linear sucrose gradient (top) showing the size distribution of microparticles in 111B.

Figure 4. Fraction 2 of sucrose gradient (second from top) showing the size distribution of microparticles in 111B.

Figure 5. Fraction 3 of sucrose gradient (third from top) showing the size distribution of microparticles in 111B.

Figure 6. Fraction 4 of sucrose gradient (fourth from top) showing the size distribution of microparticles in 111B.

Figure 7. Fraction 5 of sucrose gradient (bottom) showing the size distribution of microparticles in 111B.

Figure 8. Histogram showing the association of the pesticides dieldrin, p,p'-DDE, endrin, heptachlor, aldrin, lindane, and o,p'-DDD with each of four fractions from linear sucrose gradients of five different lake water samples.

Figure 9. Graph plotted as relative differences in growth of a Pseudomonas sp from Lake Erie grown in the presence of p,p'-DDD either in pure chemical form or adsorbed to the clay kaolinite.

Figure 10. Graph plotted as relative difference in growth of Pseudomonas sp. from Lake Erie grown in the presence of endrin either in pure chemical form or adsorbed to the clay kaolinite.

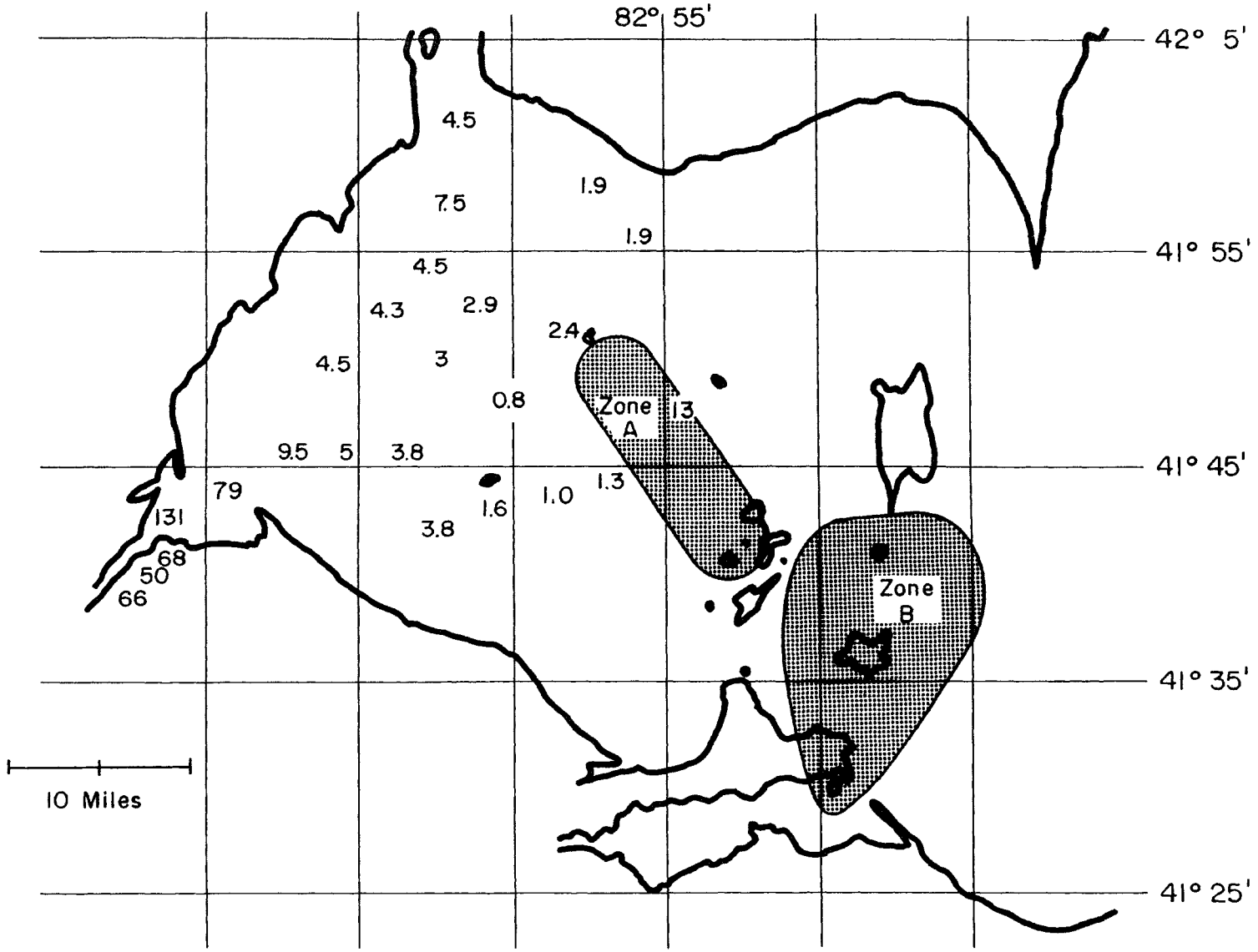



Figure 1

Map of Western Basin of Lake Erie, showing areas of study. (Zone A and Zone B).

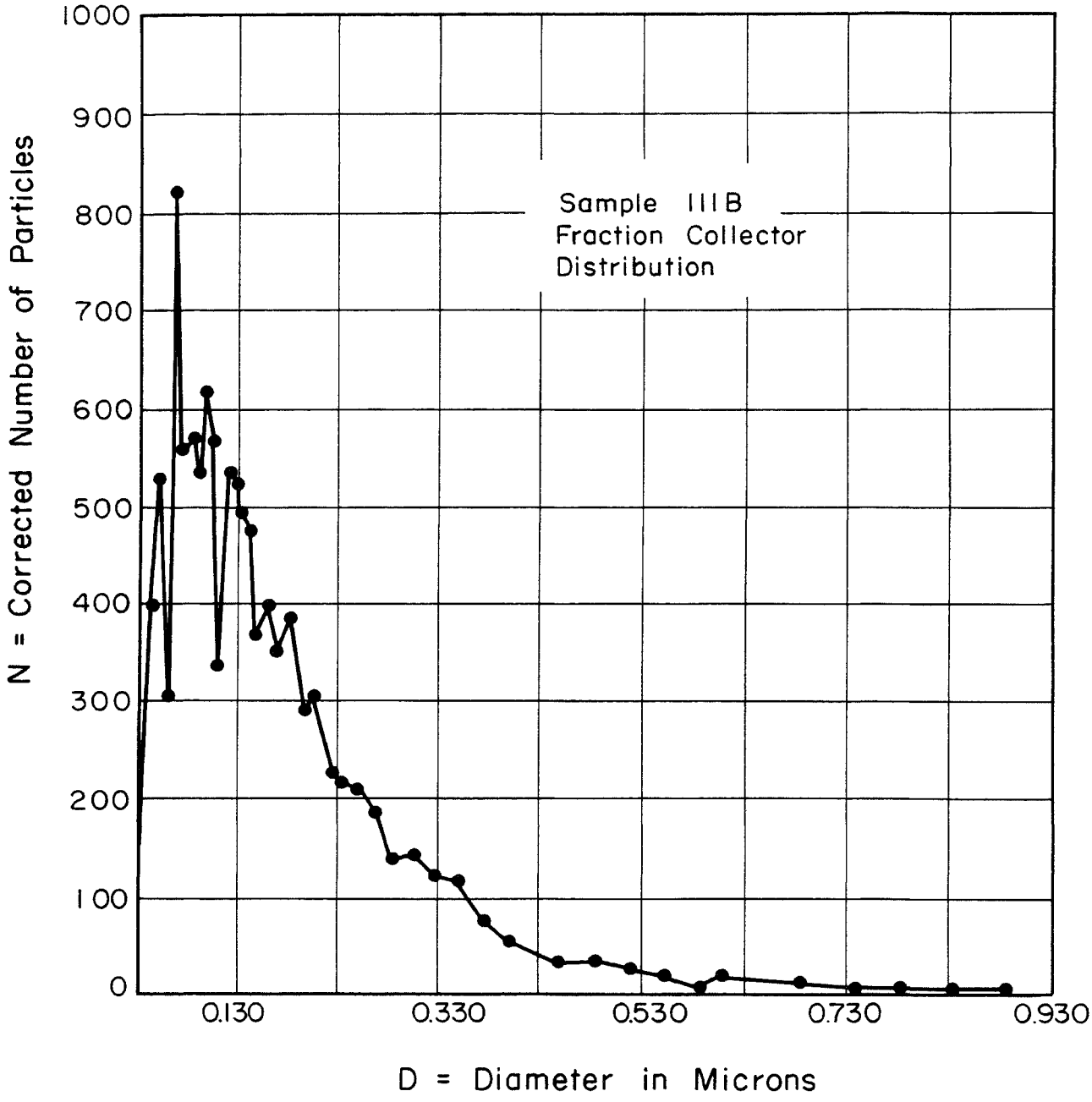

1000
900
800
700
600
500
400
300
200
100
0
N = Corrected Number of Particles
Sample 111B
Fraction Collector
Distribution
0.130
0.330
0.530
0.730
0.930
D = Diameter in Microns

Figure 2

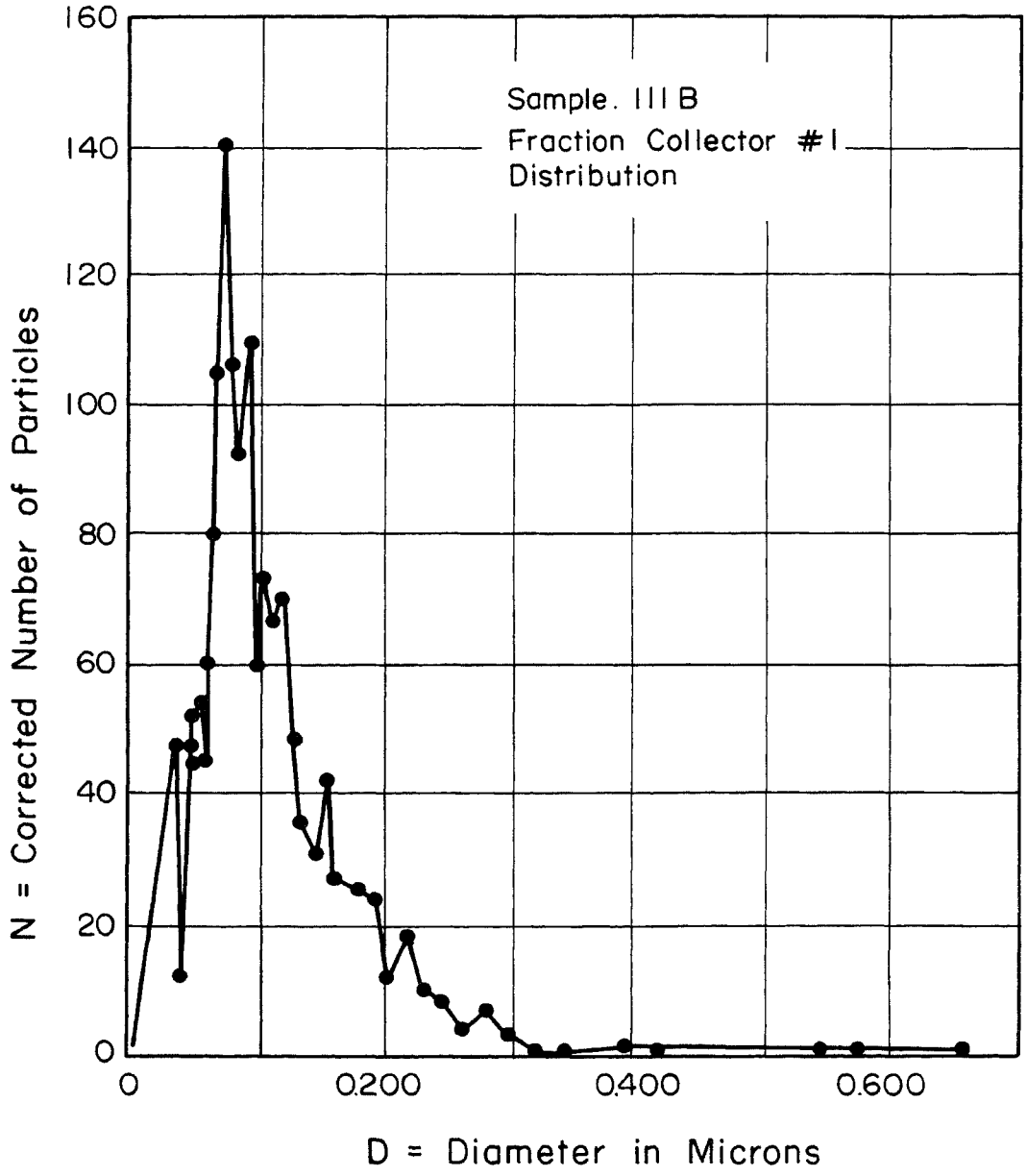

Sample. 111 B
Fraction Collector #1
Distribution
N = Corrected Number of Particles
D = Diameter in Microns
0
20
40
60
80
100
120
140
160
0
0.200
0.400
0.600

Figure 3

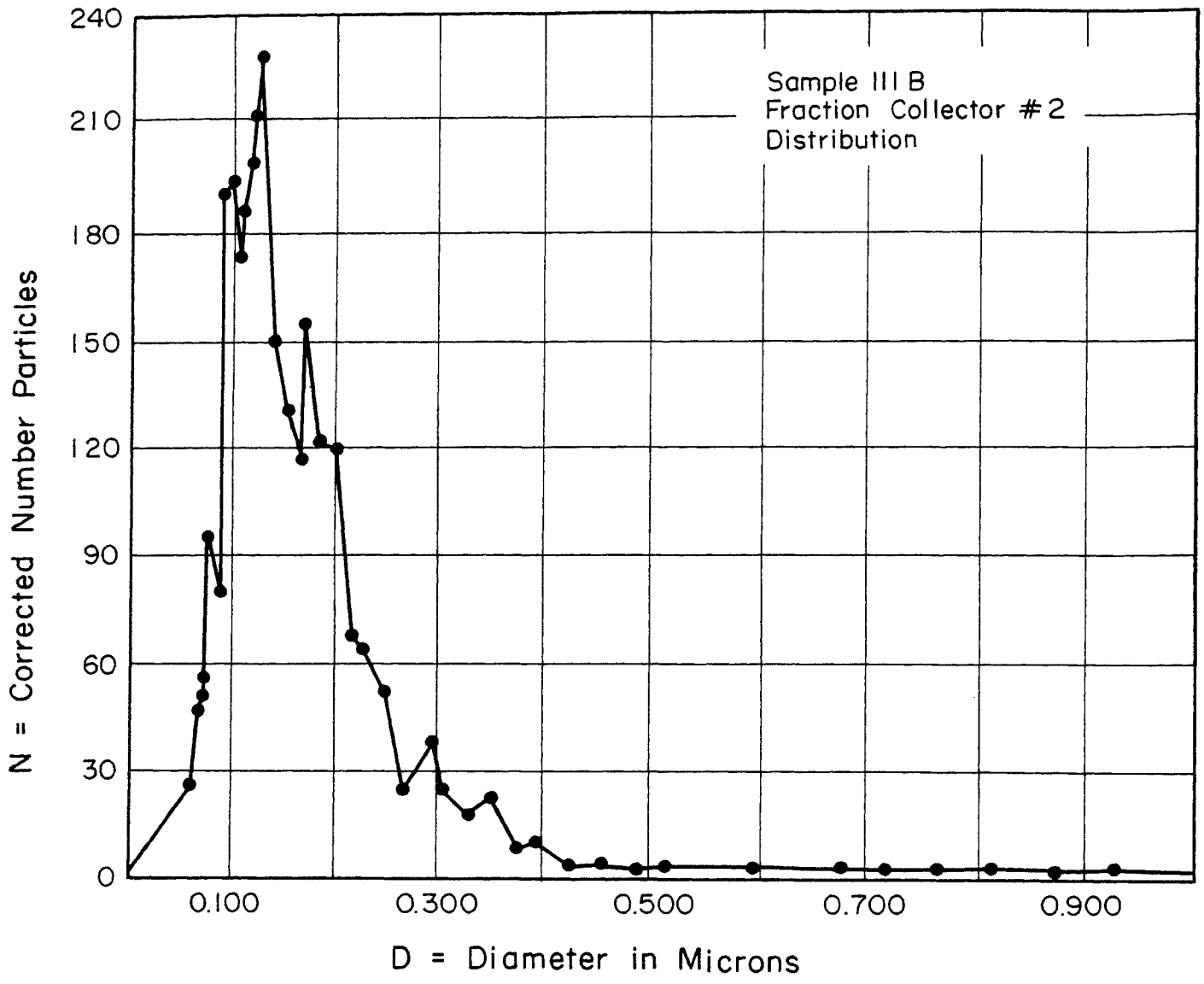

Sample III B
Fraction Collector #2
Distribution
N = Corrected Number Particles
D = Diameter in Microns
240
210
180
150
120
90
60
30
0
0.100
0.300
0.500
0.700
0.900

Figure 4

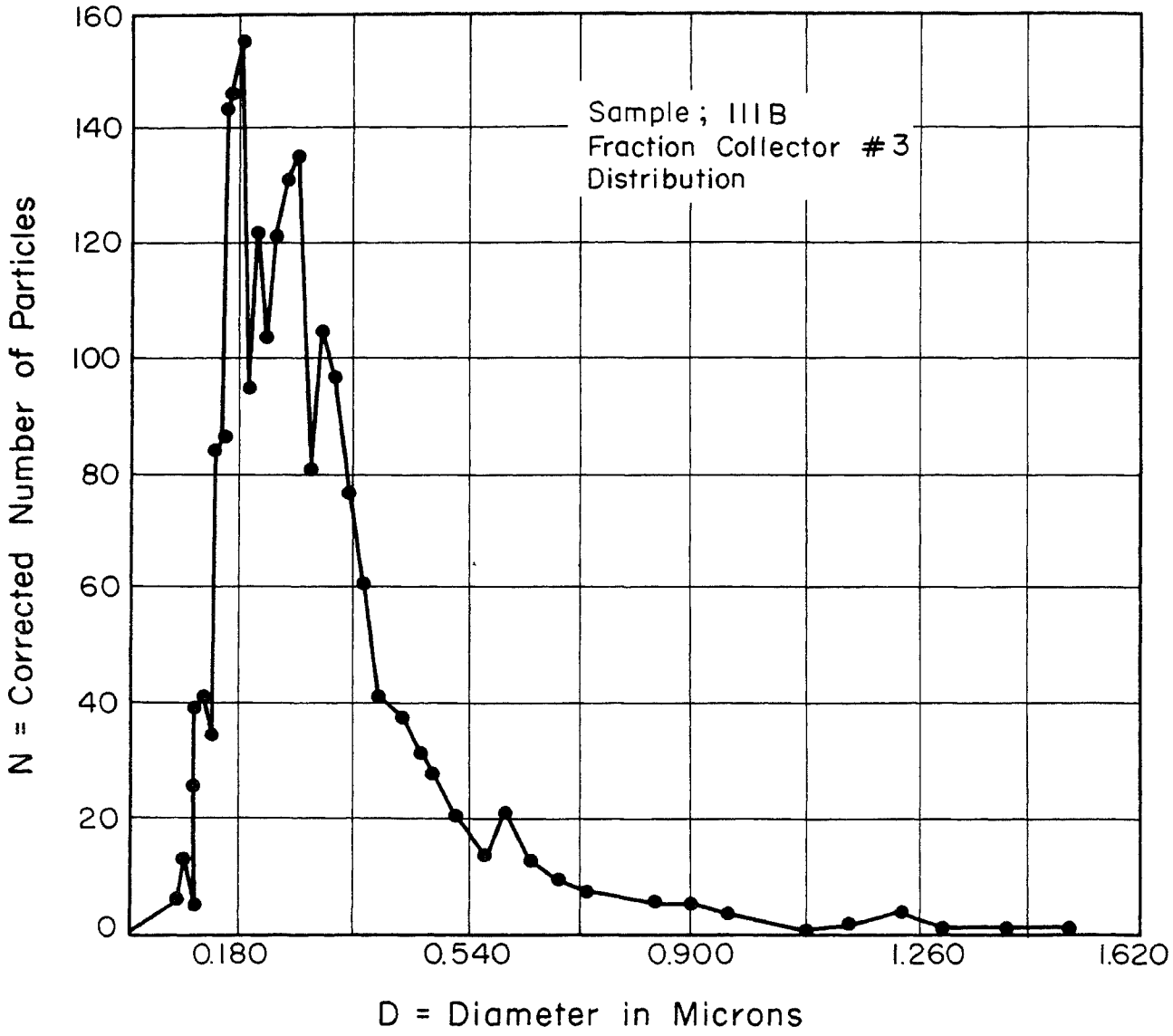

Sample ; 111B
Fraction Collector #3
Distribution
N = Corrected Number of Particles
D = Diameter in Microns
0
20
40
60
80
100
120
140
160
0.180
0.540
0.900
1.260
1.620

Figure 5

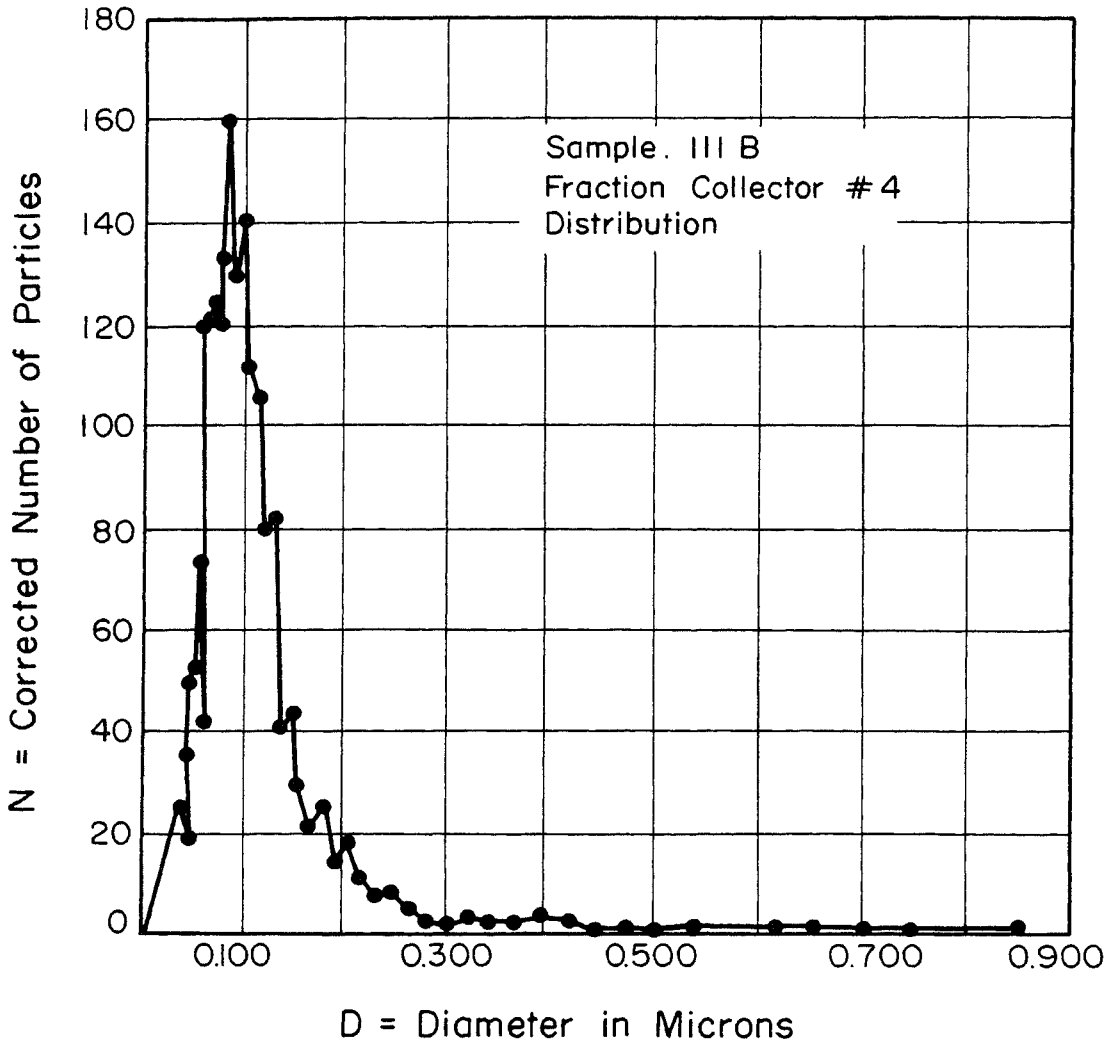

Sample. III B
Fraction Collector #4
Distribution
N = Corrected Number of Particles
D = Diameter in Microns
180
160
140
120
100
80
60
40
20
0
0.100
0.300
0.500
0.700
0.900

Figure 6

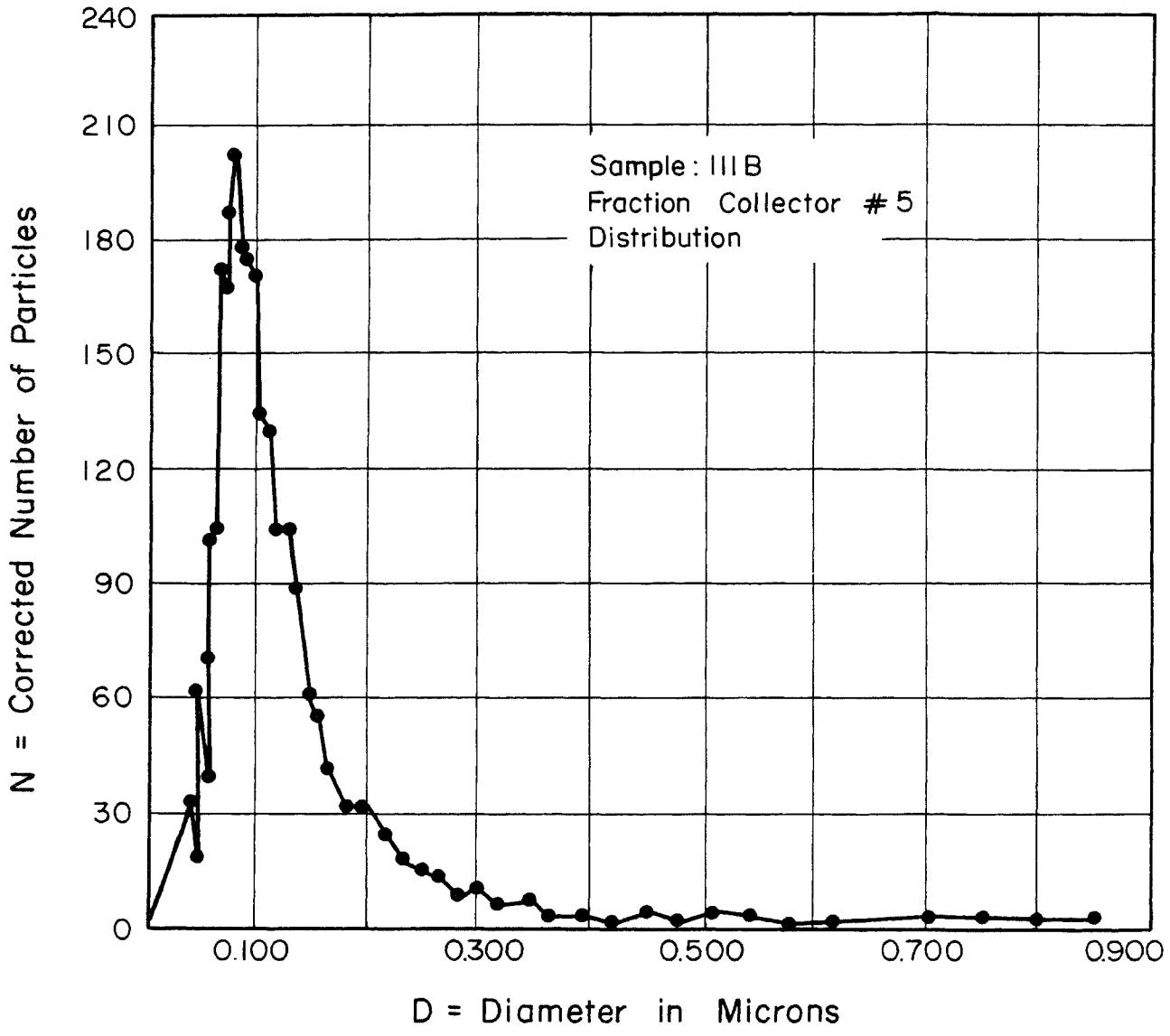

240
210
180
150
120
90
60
30
0
N = Corrected Number of Particles
Sample : III B
Fraction Collector # 5
Distribution
0.100
0.300
0.500
0.700
0.900
D = Diameter in Microns

Figure 7

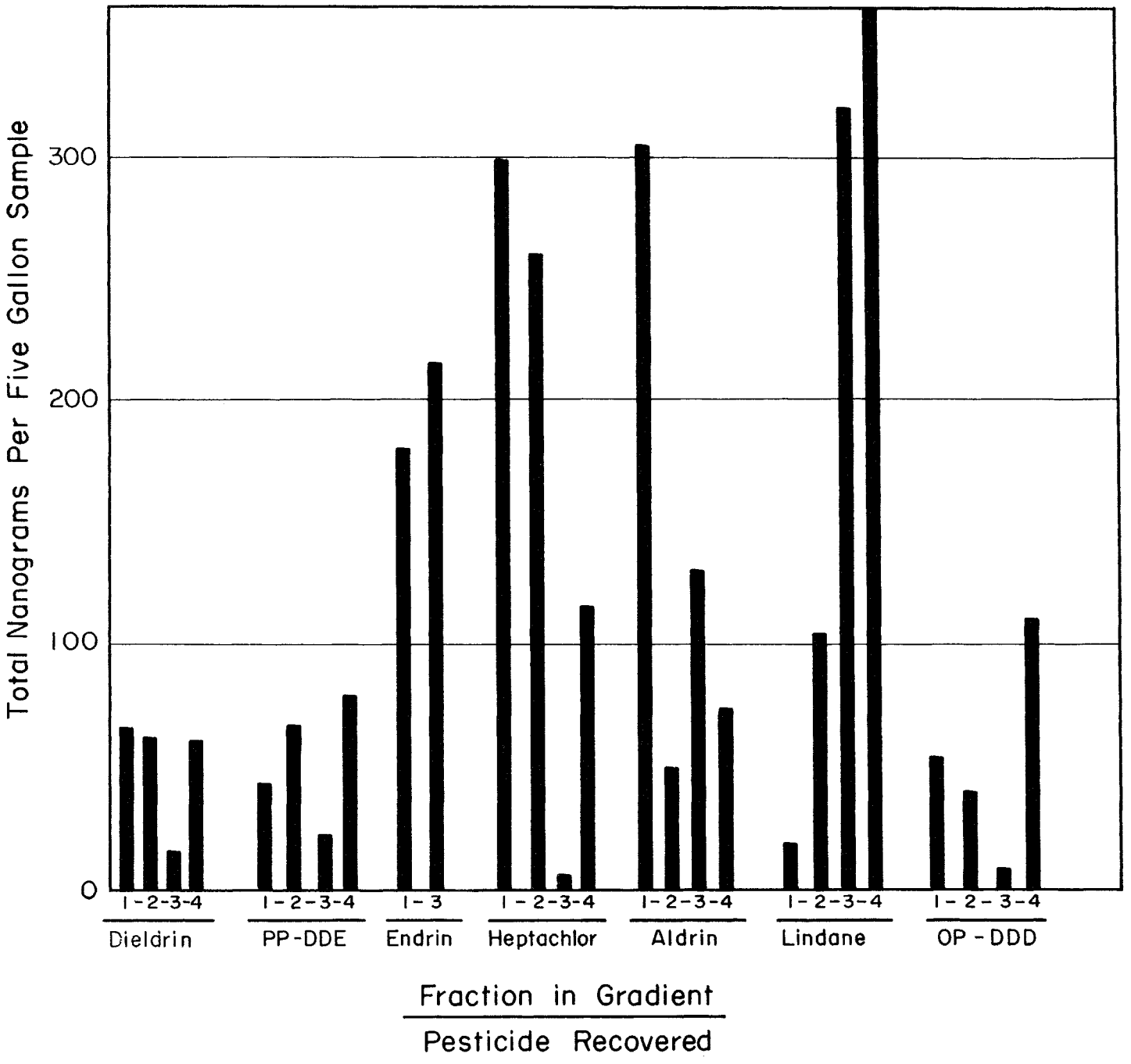

Total Nanograms Per Five Gallon Sample
300
200
100
0
1-2-3-4
Dieldrin
1-2-3-4
PP-DDE
1-3
Endrin
1-2-3-4
Heptachlor
1-2-3-4
Aldrin
1-2-3-4
Lindane
1-2-3-4
OP-DDD
Fraction in Gradient
Pesticide Recovered

Figure 8

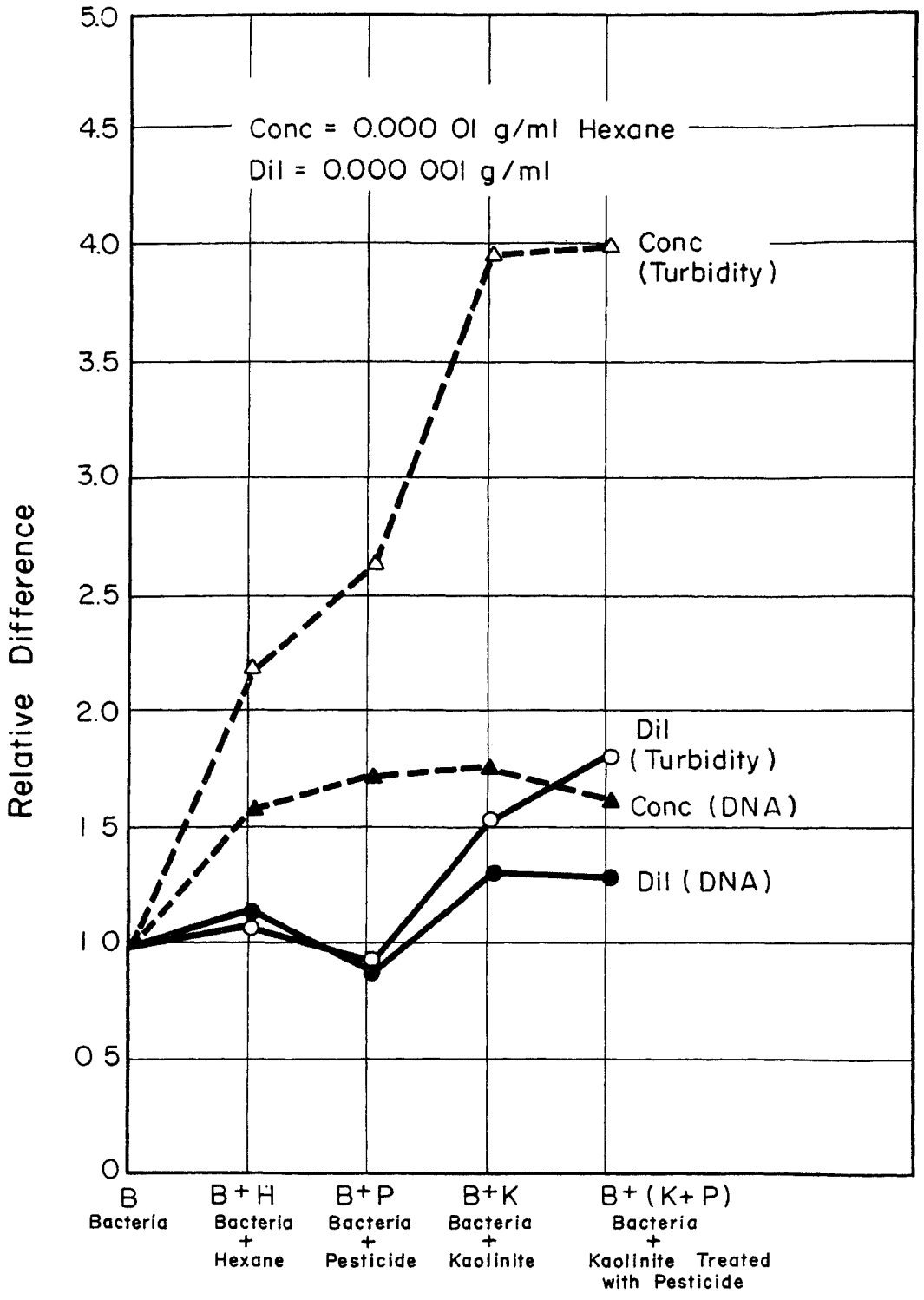

5.0
4.5
4.0
3.5
3.0
2.5
2.0
1 5
1 0
0 5
0
Relative Difference
Conc = 0.000 01 g/ml Hexane
Dil = 0.000 001 g/ml
Conc
(Turbidity)
Dil
( Turbidity )
Conc (DNA)
Dil ( DNA)
B
Bacteria
B + H
Bacteria
+
Hexane
B+P
Bacteria
+
Pesticide
B+K
Bacteria
+
Kaolinite
B+ (K+ P)
Bacteria
+
Kaolinite Treated
with Pesticide

Figure 9

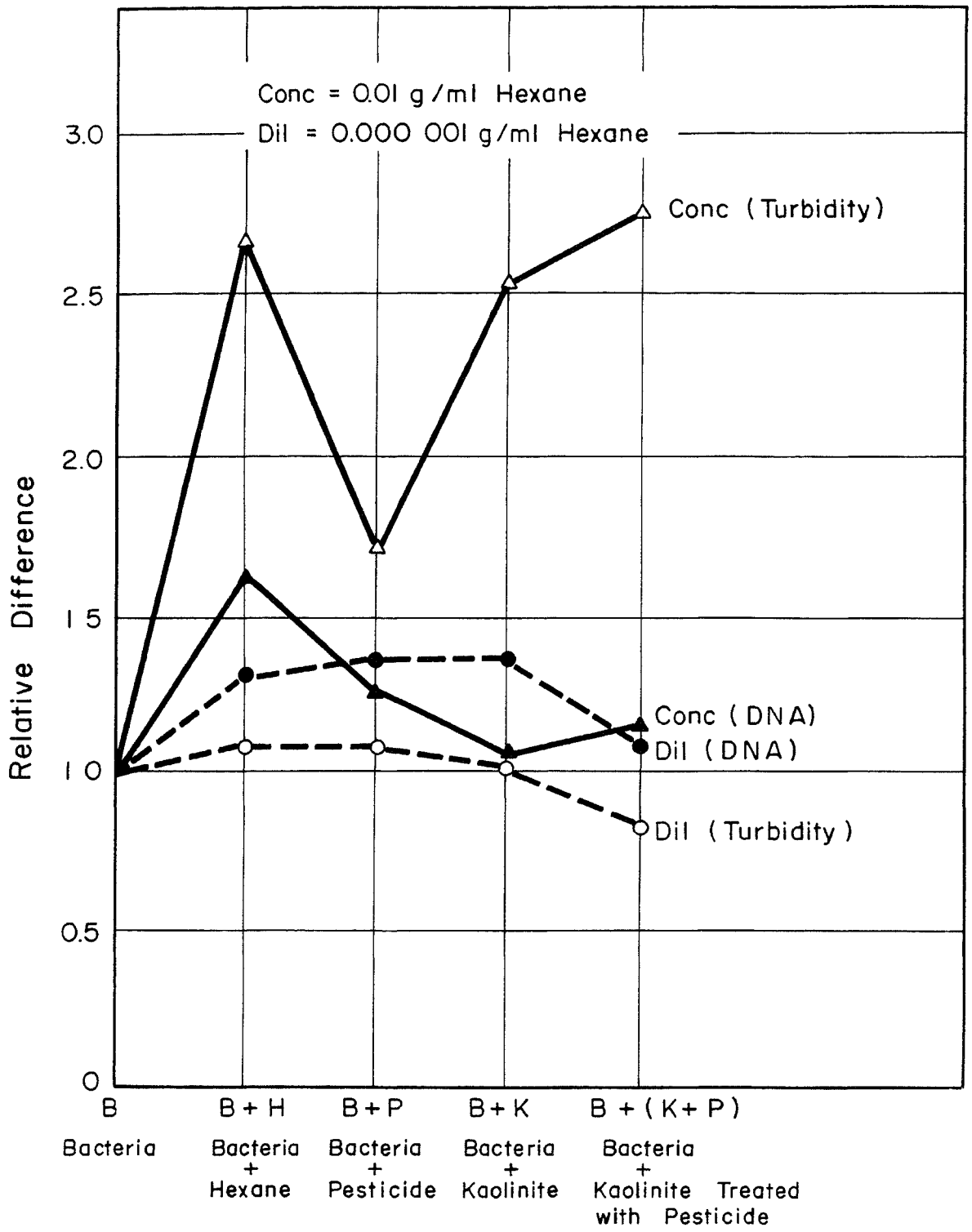

Conc = 0.01 g/ml Hexane
Dil = 0.000 001 g/ml Hexane
Conc (Turbidity)
Conc (DNA)
Dil (DNA)
Dil (Turbidity)
Relative Difference
3.0
2.5
2.0
1.5
1.0
0.5
0
B
Bacteria
B + H
Bacteria + Hexane
B + P
Bacteria + Pesticide
B + K
Bacteria + Kaolinite
B + (K + P)
Bacteria + Kaolinite Treated with Pesticide

Figure 10

Microbial and Chemical Interactions in Lake Erie. A Summary Statement.

During the last conference devoted to the subject of a systems approach to Great Lakes water resources we pointed out that the type of biological-chemical-physical data which was required for a systems type evaluation of the Great Lakes environment was different from the kind of data required for a systems approach directed toward water management. That is, interractions in the aquatic ecosystem are dictated by inflexible physical and chemical laws whereas inputs to management type systems analysis are usually based upon more flexible social laws and decisions.

It then became apparent that we had neither sufficient specific data nor the necessary understanding of interractions in the aquatic environment to predict what reaction to expect as the result of altering some of the recognizable variables. We could, however, predict that some type of secondary interractions would occur as the result of altering any variable in the environment.

It is also apparent that policy and management decisions pertaining to Lake Erie, which is undergoing eutrophication at an accelerating rate, cannot wait for development of a comprehensive understanding interractions controlling the ecosystem. However, there seems to be general agreement that certain problems are of primary significance in the lake and some of the related interactions can be identified at the present time (Dugan, 1967).

It is the intent of this report to contribute information on the ecological subsystem and to emphasize the relative significance of interactions being observed in Lake Erie, which can then be considered by others in an overall Systems Analysis (e.g. by Randles, et al, Tybout, et al.).

One of the major problems in Lake Erie is the increased growth rate of algae and other microorganisms. Blooms of the blue green algae Aphanizomenon, Anabaena, and Microcystis (Anacystis) are particularly evident in the lake and it is the blue green algae that are quite characteristic of eutrophic conditions. The observed genera are planktonic forms which generally prefer warmer, more alkaline water and tend to grow more rapidly than most green algae. For example: Species of Anabaena and Microcystis have been reported to have a mean generation time of 10.6 hours and 2.0 hours respectively under ideal conditions (Fogg, 1966).

Some of the objections to increased concentrations of blue green algae in the lakes are: (a) the aesthetic repulsion to the presence of green slime in water by the recreation minded public; (b) the potential loss of property value related to decreased recreational value; (c) the potential toxic factors produced by blue green algae which can result in mortality of fish and domestic animals (Shilo, 1967); (d) clogging of water supply intake filters; (e) depletion of $O_2$ in water as the algal blooms decompose.

In order to draw on experimental evidence which is available in the literature pertaining to plant growth, we wish to emphasize the similarity of basic ecological considerations in soil and water, particularly with regard to nutritional effects of fertilizer on plant crops and algal cells.

The bloom producing blue green algae in Lake Erie require relatively little nutrient for growth. Several species which have been observed this summer (1969) (Anabaena, Nostoc, Aphanizomenon) have been implicated in the process of converting atmospheric nitrogen ($N_2$) into a form which is utilizable for growth by themselves and other microorganisms. That is, these organisms fertilize the lake with nitrogenous nutrients. In addition to nitrogen, the blue green algae require carbon dioxide (dissolved as carbonate in alkaline water), light as an energy supply and a few mineral salts (e.g. $PO_4$, $SO_4$, Mg, Ca, Fe, B, Mo, Mn, Na, Co, Cu and Zn). To our knowledge no blue green algae have been shown to grow heterotrophically (i.e. use organic compounds for energy in place of photosynthesis), although they are known to photoassimilate 18 to 32 percent of their cell weight from organic compounds such as acetate, glucose, amino acids, urea, casein and certain other chemicals. Once an algal bloom has formed, the algae are capable of excreting substantial amounts of amino acids, peptides and polysaccharides into the surrounding water.

The net effect of the presence of blue green algae is to increase fertility of the water, particularly by forming a reservoir of reduced carbon (organics) which can be used by bacteria, eucaryotic algae (e.g. green algae), and higher plants.

Four general parameters must be considered in relationship to accelerated growth of blue green algae: (1) amount of light energy); (2) nitrogen supply ($N_2$, $NH_4$, $NO_3$, $NO_2$, urea, amino acids, etc.); (3) $CO_2$ or $CO_3$ and (4) minerals.

Some of the data we have collected allows us to make several observations in this regard. Figure 1 presents data collected in the western basin of Lake Erie (Figure 1 Part 4) during the spring and

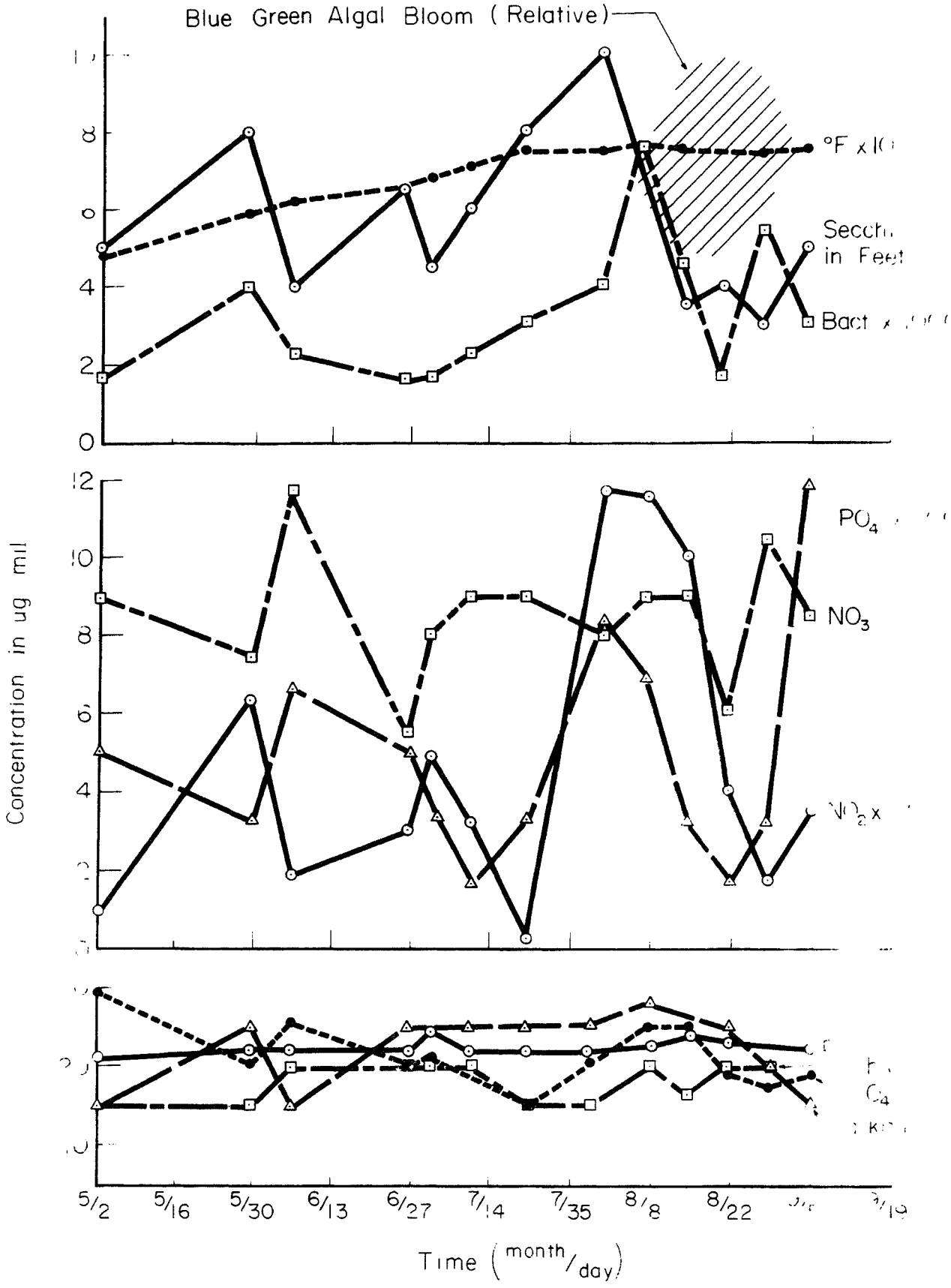
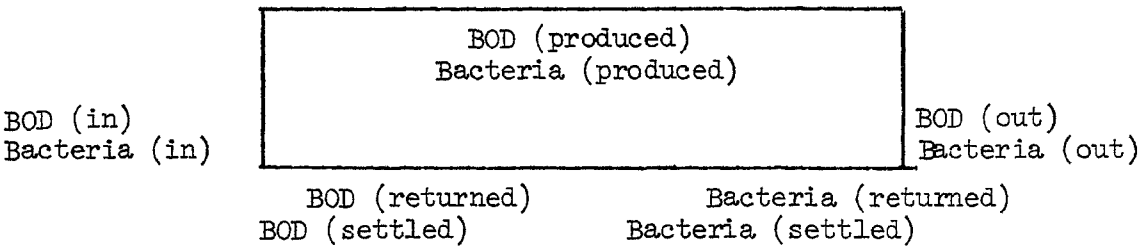

Blue Green Algal Bloom ( Relative)
°F x 10
Secchi in Feet
Bact x
PO4
NO3
NO2 x
Concentration in ug ml
5/2
5/16
5/30
6/13
6/27
7/14
7/35
8/8
8/22
Time (month/day)

**Figure 1**

summer of 1969. All analyses shown were obtained on water samples taken at a depth of 15 feet.

If one assumes fairly uniform inputs and outflows from the lake of phosphorus, nitrogen, carbon, other minerals, and a uniform input of light, then the entrapment and accumulation of the chemicals must depend on mechanisms which convert, and recycle, and finally deposit them in the lake. Thus a nutrient input may be biologically channelled through several cycles of fixation and release before it effectively leaves the system. For example, it has been estimated that a biochemical oxygen demand (BOD) of $540 \times 10^6$ lbs of oxygen consumption in the form of organic inputs enters the lake in a year; yet the estimated real BOD of the organic materials in the lake for a year is 18 times greater than the input organic demand (FWPCA report 1968). It is therefore likely that the original input is being recycled through bacterial decomposition followed by resynthesis by algae and other microbes.

The recycling can be pictured as shown diagrammatically below:



An overall equation can be written indicating the relationship of bacterial action to the recycling. This equation is:

$$BOD_i + BOD_p + BOD_r - BOD_o - BOD_s = k\ (bacteria_p)$$

where:

$BOD_i$ equals BOD entering system

$BOD_p$ equals BOD produced in system

$BOD_s$ equals BOD removed from system by settling of insoluble material (microbial cells, organic particulates)

$BOD_r$ equals BOD resuspended by mixing and solubilization by microbial action

$BOD_o$ equals BOD leaving the system via the outlet or via degradation

$bacteria_p$ equals bacterial mass produced on the available substrate

k equals a constant relating bacterial mass produced per mass of oxygen and depending on the nature of the substrate

BOD could be replaced by bacterial mass if we assume that for every pound of bacteria produced aerobically, between 1 and 5 pounds of oxygen are required depending on the substrate (Johnson, 1967). On glucose and similar substrates, the oxygen requirement is such that 1 pound of oxygen is required to produce 1 pound of bacterial cells. Assuming that glucose fairly represents the utilizable organics in the system, then a BOD of 1 will allow the production of 1 pound of bacterial mass. Thus, if we determine the instanteous mass of bacteria and if we know the generation time (doubling time) of the bacterial population we can estimate the real BOD present in the lake in a given time interval.

Since the total annual BOD of the lake is estimated to be 18 times the input BOD from discharges into the lake, then the bacterial mass that could be produced is 18 times that accounted for by input of bound carbon. This would suggest that between 4 and 5 effective generations

occur in a year giving a generation time of between 2 and 3 months. Generation times of bacteria are considerably shorter. For example, Jannasch (1969) has reported generation times in sea water of 53-60 hours for 2 different bacteria and of 140 hours for a third - suggesting that a good deal of carbon turnover occurs by cycling through the bacterial population. In this regard, Lake Erie is both nutritionally richer and warmer than the sea and shorter generation times would be expected. Actual calculations based on our aerobic heterotrophic bacterial counts show a minimum of $1 \times 10^8$ lbs. bacteria in the lake on the average as compared to a possible $9.7 \times 10^9$ lbs based on total available BOD.

For every unit mass of bacterial cells produced about 3 lbs of glucose is utilized and 3 lbs of $CO_2$ is released. Thus, the incoming organic content must be 3 times the BOD. If an estimated there is in the lake 18 times the input BOD then the total added bound carbon expressed as glucose must be of the order of 54 times the input BOD. On the assumption that eventually all of the bound carbon in the input becomes $CO_2$ through several successive steps of bacterial action, then the total available $CO_2$ from incoming organics amounts to nearly 1.5 times the total weight of the input organics.

If completely efficient recycling were to occur and all of the $CO_2$ came from organic inputs then 18 complete cycles of the carbon would be necessary to generate the estimated BOD. Complete recycling does not occur because there is a build up of organic carbon both in the sediments and bound in microbial cells. In fact, the carbon bound in bacterial cells suspended in the lake at any one time amounts to $5 \times 10^7$ lbs of a potential BOD of $1 \times 10^8$ lbs of oxygen, which constitutes about 20 percent of the annual BOD input from discharges to the lake. These

are suspended bacteria but about 1/3 of the bacteria and many of the algae are floc formers which may aggregate and settle; taking with them carbon, nitrogen, phosphorus, and adsorbed trace minerals.

Herdendorf (1968) reported that contemporary sedimentation was greater than anticipated on the basis of bottom sediment accumulation and that an increase in organic content of sediments corresponds to periods immediately following plankton blooms.

In the highly reducing muds, the settled organic material is anaerobically degraded to yield $CO_2$, soluble organics, minerals and a host of reduced compounds such as $CH_4$, $N_2$, $H_2S$, $H_2$, and CO. Thus is stimulated further activity by aerobic bacteria and by the algae. The production of $CO_2$, soluble organics and soluble minerals from insoluble biological residues in the mud may be a key feature in triggering algal blooms. If $CO_2$ were limiting, then the $CO_2$ produced by the anaerobes in the mud and the $CO_2$ produced by the aerobes from the soluble organics arising from anaerobic decomposition of resistant organics in the mud could act as a triggering supply of $CO_2$. That a significant amount of anaerobic activity occurs in the bottom muds has been documented by our discovery of significant methane evolution and a measurable nitrogen fixing capacity in the muds.

An increase of $7 \times 10^3$ bacteria per ml, as occurred in a three week period this summer, represents a net increase in bacterial bound carbon of $1.6 \times 10^9$ gms in the lake. This increase in bacterial mass provided in the same interval a BOD of $7.1 \times 10^6$ lbs. of oxygen and utilized $2.1 \times 10^7$ lbs of glucose equivalent. In the growth of these bacteria about $7.1 \times 10^6$ lbs of glucose equivalent was converted to microbial cells and $2.1 \times 10^7$ lbs of $CO_2$ was released. This same mass of bacterial cells would bind $5.8 \times 10^5$ lbs of nitrogen (or $2.7 \times 10^6$ lbs expressed as nitrate) and $7.1 \times 10^4$ lbs of phosphorus (or $2.2 \times 10^5$ lbs expressed as phosphate) in the cells themselves. Thus, the increase of bacterial mass in itself represents a significant factor in the availability of nutrients. Conversely, the metabolic activity associated with such a mass increase would result in the release from bound sources of significant quantities of materials such as phosphorus, nitrogen, and trace minerals which could stimulate algal growth.

Bacteria which are present in soil but which are not intimately associated with plant cells, are known to be able to solubilize nutrients and make them available for plant growth. For example, $PO_4$ can be released from "insoluble" mineral forms such as $CA_3(PO_4)_2$, $Fe_3(PO_4)_2$ and $AlPO_4$. It is known that other bacteria which are intimately associated with plant cells (e.g. embedded in the mucigel layer of root cells or in the microfibrillar network of cell walls) will adsorb $PO_4$ at the expense of the plant cell. The $PO_4$ is incorporated into bacterial protoplasm where it is unavailable to the plant cell until the bacteria disintegrate (Barber, 1968). This phenomenon is significant when the $PO_4$ concentration is less than 0.3 ppm, as in the case of Lake Erie. Since bacteria are also intimately associated with algal cells, it may

a role in governing rates of algal growth or blooms.

We have also taken a crude measure of the total amount of algae present in Lake Erie water just after the algal bloom peaked out (September 1, 1969) and found an average of 0.041 g (dry wt) of algae/L. (Material retained by a 1.2 u millipore filter). This is equivalent to approximately $4 \times 10^{11}$ lbs algae (dry wt) in the lake during a bloom.

The ratio of phosphorous to nitrogen to carbon in algal cells is reported to be 1:16:100 on a molecular basis (Megregian, 1967) or a ratio of about 1:7:40 on a weight basis. If we assume that the dry weight of algal cells is 40 percent carbon we can say there was (.041 g) or .0164 g of carbon per liter of Lake Erie water tied up in the algal cells, which is equivalent to 16 ppm. The nitrogen would be 7/40 (.0164) - approximately 0.003 g/l = 3.0 ppm and the phosphorous would be 1/40 (.0164) = approximately 0.0004 g/l = 0.4 ppm.

These values can be compared to the values presented in Figure 1. It can be seen that 0.2 ppm $PO_4$ was removed from the water column during the algal bloom (7/31 to 8/21) and reduced the lake concentration to 0.05 ppm. During this time 3.0 ppm nitrate was removed but 6 ppm was still available. Total alkalinity (a measure of carbonate-bicarbonate) fluctuated about 5 ppm but 90 ppm was still available.

This indicates that although 16 parts of carbon per million parts of water was tied up in algal cells, only about 5 ppm was missing from the water columns. Therefore we again must conclude that additional carbon must be entering the system during algal growth. As we have already pointed out, additional $CO_2$ can come from either anaerobic degradation of sediments or aerobic respiration or diffusion from the atmosphere.

The data also suggest that during the algal bloom $PO_4$ may have become limiting. The weight of algae would contain an equivalent of 0.4 ppm bound $PO_4$ but during this period algae removed 0.2 ppm from the water column and only 0.05 ppm remained in the water. However, $PO_4$ was rapidly going into solution just prior to the algal bloom and this correlated with an increase in bacterial numbers. There is no reason to assume that solubilization of $PO_4$ via bacterial action ceased while the algae were removing it, and this could account for the greater total amount in algal cells than was available at any instant in the water column. Sufficient $NO_3$ and carbonate remained in solution to support an algal demand for an additional 0.8 ppm $PO_4$.

It is also known that algal cells synthesize polysaccharide slime from carbon when the ratio of carbon to $PO_4$ and $NO_3$ is high (Holm Hansen, 1968). This is the case during the algal bloom peak and might explain the decrease in alkalinity after 8/20 (Figure 1).

Another physical-biological relationship that we believe is quite significant is the association of pesticides with microscopic particulate matter in the lake. We have been able to show that pesticides are held in suspension via adsorption to very small particles (Pfister, et al, 1969) and that these particles can in turn be adsorbed by microorganisms (bacteria

and algae) (Pfister, et al, 1969b). It is this conglomerate of interacting particles that make up the sediment coming out of the water column. During periods of low bottom turbulence the sediment which is high in pesticide as well as inorganic minerals will accumulate on the bottom. This forms a plausable basis for explaining the loss of pesticide susceptible insect larvae and its subsequent influence on dependent fish population or other organisms in a food chain. In this regard, we are aware that Chironomidae increased in number in the bottom sediment when Hexagenia decreased during the 1955-1960 period of national pesticide onslaught (Dambach, 1969). We are unaware of the relative susceptibility of these particular organisms to chlorinated pesticides; however, it is known that other groups of organisms display a range of susceptibility to any given pesticide. This rational is also reasonable if pesticide effects in fish are direct rather than through a food chain. For example, pike are more susceptible than carp to most chlorinated hydrocarbons.

It must be emphasized that we have made many assumptions in our calculations. However, the theoretical calculations compare favorably with calculations based upon our data. We are also aware that we have not considered total phytoplankton and zooplankton in the water column which will have to be considered and integrated with out data as our efforts progress.

Summary

Decision making activites are best carried out when a complete description of the lakes environmental system is available. It is not likely that such a description will be forthcoming in the immediate future, but its lack should not prevent description and action based on key parameters that can be presently assessed.

We cannot expect to obtain representative relationships within the lake by simple equation of chemical data in the water column (e.g. dissolved chemicals) to gross observable effects on a 1:1 basis. Interrelationships are more complex.

Primary productivity can be measured and its impact on the biology and chemistry of the lake can be approximated. Similarily, the bacterial productivity can be estimated and its role and impact in nutrient removal and turnover can be assigned some relative importance. Further, the nature and activity of heterotrophic bacteria in the water and bottom muds can be estimated. These estimates and assessments can be used in systems analysis for their predictive capabilities. For example, the measurement of nitrogen fixing activity may indicate some reduction in the need for nitrogen in other forms, thus the critical level of nitrogen in other forms would have to be lowered. Similarly the production of methane effectively removes some of the carbon from the system in a form which will not be recycled by algae.

Although buildup of flocculent biological material aids in sedimentation of suspended particulates and colloidal substances (organic + inorganic, toxic + nutritious), the sediments ultimately contribute to algal growth via recycling mechanisms, and probably to reduction of fish populations either directly or via reduction in insect forage.

If all organic and mineral inputs to the lake are stopped, the direct effect will be decreased algal and bacterial populations. However, the sediments already deposited in the lake will contribute significantly to algal growth until the sediments are either biologically lowered in organic content or until they leave the lake. Therefore, rates of sediment formation and removal must be determined.

The role of bacteria in making nutrients available for algal growth is significant: and algae, once above a critical concentration, significantly enrich their own environment with organics, which indicates a spiraling increase in rate of eutrophication, not unlike the forces of inflation.

These factors imply:

(1) That the organic nutrients upon which bacteria grow must either be prevented from entering the water column or the bacteria must be inhibited in the lake.

(2) Mineral nutrients upon which algae grow must be prevented from entering the water column.

(3) Sediments of relatively high organic content must be removed or allowed to decrease naturally, a process which will determine the rate of decrease of the eutrophication rate.

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